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# *STUDIES ON THE BIOCHEMISTRY OF TETRAHYMENA. IX. FOLIC ACID COMPONENTS AND CONJUGATES**

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*Introduction.*—The recent publication of the reports of the conference on folic acid[1] has served to clear up many confusing points in the literature on this subject. It is possible now to substitute chemical terms for such names as "folic acid," "Vitamin $B_c$," "*L. casei factor*," "norit eluate factor," "Vitamin M," etc., which are considered synonymous with pteroylglutamic acid; "fermentation folic acid," which is pteroyldiglutamyl glutamic acid; and "Vitamin $B_c$ conjugate," which is pteroylhexaglutamyl glutamic acid. These three members of the "folic acid group" possess widely different activities for the lactic acid bacteria but, so far as the investigations have gone, similar activities for animals.

The discovery that the animal microörganism, *Tetrahymena geleii*, requires pteroylglutamic acid for growth[2] makes it of importance and interest to investigate the activity of the related compounds in the growth of this organism. Because of the fact that the metabolism of *T. geleii* has been shown to be more typically animal-like than that of any other microörganism so far studied,[3] it might be suspected that the two conjugates would prove active. Bacteria[4] differ from typical animals (chick, rat, monkey[5]) in what has been interpreted to be a lack of specific enzymes for degradation of the conjugated compounds. It has been found also that humans suffering from pernicious anemia, unlike normal humans, are unable to utilize the conjugates,[6] and this has also been attributed to a lack of conjugase.

*Material and Methods.*—The organism used in this investigation was the ciliated protozoan, *Tetrahymena geleii* W, grown in pure (bacteria-free) culture.[3] Dose-response tests were carried out as previously described.[2, 7] The base medium has been modified in respect to the amino acid concentrations from that which was formerly used,[2, 7] as our investigations (still

in progress) have shown that better growth can be obtained by such changes. The complete base medium is given in table 1. It is to be noted that both *p*-aminobenzoic acid and inositol have been omitted from this medium, since the organism has been found to be capable of synthesizing these compounds and their inclusion in the medium is without effect.[8] Guanylic acid has been substituted for guanine because of the greater solubility of the former. Brucine cytidylate was used in place of cytidylic acid because our supply of the free acid was exhausted. Comparative tests of these last compounds showed no indications of toxicity of the brucine salt.

TABLE 1

BASE MEDIUM

| | γ/ML. | | γ/ML. |
|---|---|---|---|
| *l*(+) Arginine Monohydrochloride | 125 | Biotin (free acid) | 0.0005 |
| *l*(−) Histidine | 125 | Calcium pantothenate | 0.10 |
| *dl*-Isoleucine | 125 | Thiamine Hydrochloride | 1.00 |
| *l*(−) Leucine | 250 | Nicotinamide | 0.10 |
| *l*(+) Lysine | 250 | Pyridoxine Hydrochloride | 0.10 |
| *dl*-Methionine | 500 | Riboflavin | 0.10 |
| *dl*-Phenylalanine | 350 | Choline chloride | 1.00 |
| *dl*-Serine | 250 | Guanylic Acid | 25.00 |
| *dl*-Threonine | 125 | Adenylic Acid | 25.00 |
| *l*(−) Tryptophane | 50 | Brucine cytidylate | 50.00 |
| *dl*-Valine | 125 | Uracil | 50.00 |
| Dextrose | 1000 | Factor II preparation from liver 1:10 (prepared as previously described[3]) | |
| $MgSO_4.7H_2O$ | 100 | | |
| $K_2HPO_4$ | 100 | | |
| $CaCl_2.2H_2O$ | 50 | | |
| $FeCl_3.6H_2O$ | 1.25 | | |
| $MnCl_2.4H_2O$ | 0.05 | | |
| $ZnCl_2$ | 0.05 | | |

Pteroylglutamic acid, pteroic acid, pteroyldiglutamyl glutamic acid (fermentation factor) and 2 amino-4 hydroxy-6 methyl pteridine were supplied by Dr. E. L. R. Stokstad of Lederle Research Laboratories. Xanthopterine and *p*-aminobenzoyl-glutamic acid were supplied by Dr. George H. Hitchings of the Wellcome Laboratories. The sample of pteroylhexaglutamylglutamic acid (Vitamin $B_c$ conjugate) was sent to us by Dr. O. D. Bird of Parke, Davis Co. Research Laboratories. Without the generous coöperation of these investigators this study would not have been possible.

Since the terminology of the group of compounds under discussion is rather cumbersome, a set of abbreviations similar to that used by Lampen and Jones,[9] has been devised. These are as shown in table 2.

TABLE 2

| | |
|---|---|
| PAB | *p*-Aminobenzoic Acid |
| PGA | Pteroylglutamic Acid |
| PABG | *p*-Aminobenzoylglutamic Acid |
| PHGA | Pteroylheptaglutamic Acid (this is, in turn, an abbreviation for pteroylhexaglutamylglutamic acid[8]) |
| PTGA | Pteroyltriglutamic Acid (Pteroyldiglutamylglutamic acid[8]) |

*Results.*—When the activity of PGA was tested, using the present base medium, it was found that half-maximum response was obtained with 0.00064 microgram per ml. of medium (Fig. 1). This is in good agreement with our earlier findings[7] of 0.00065 microgram per ml. of medium for half-maximum activity.

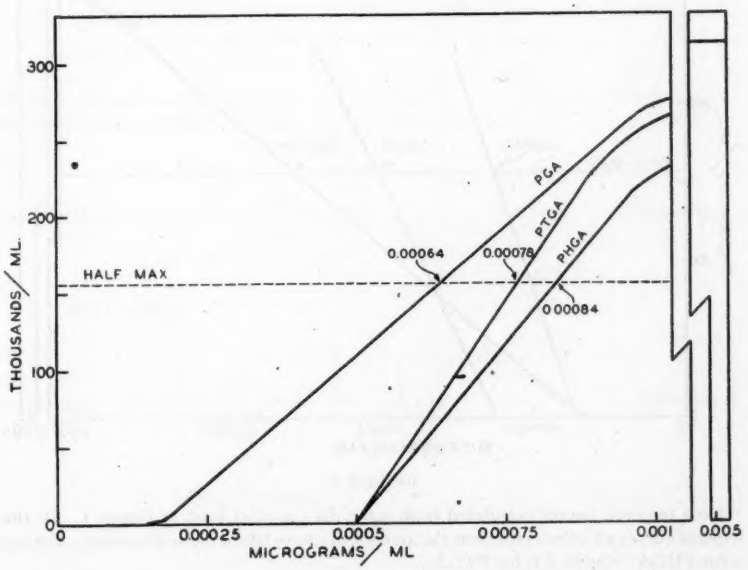



FIGURE 1.

Dose response curves of PGA, PTGA and PHGA. Dosage is on a total weight of compound basis. The curves were constructed from results obtained after third serial transplant.

Figure 1 also represents the growth response of *T. geleii* to varying concentrations of PTGA and PHGA. The half-maximum potency of PTGA is 0.00078 microgram per ml. and that of PHGA is 0.00084 microgram per ml. These responses are on the basis of the total weight of compound used. When these values are recalculated for the free PGA content, very

different curves are obtained (Fig. 2). It is immediately apparent that PHGA is more active than PTGA, which, in turn, is more active than the free acid. On the basis of PGA content, PHGA has a half-maximum potency of 0.00032 microgram per ml. This figure is just half of that which is obtained for free PGA, indicating that in the conjugated form PGA is twice as active. The half maximum activity of PTGA may be expressed as 0.0005 microgram of free PGA per ml., which is intermediate between the two.

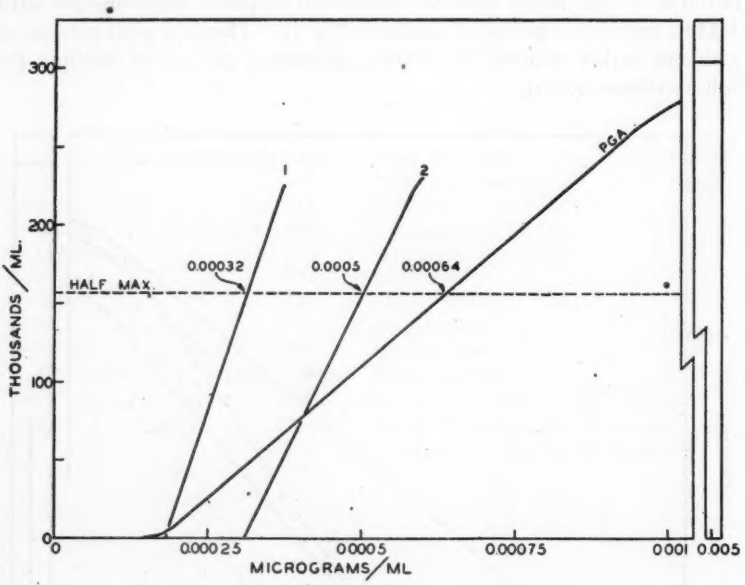



FIGURE 2.

Dose response curves calculated from same data as that used in Figure 1. In this series of curves all values represent the amount of pteroylglutamic acid present. Curve 1 is for PHGA. Curve 2 is for PTGA.

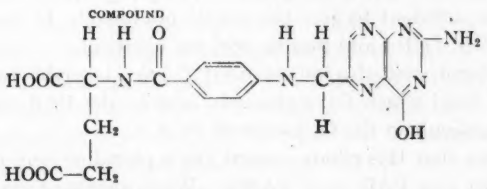
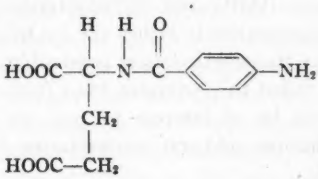
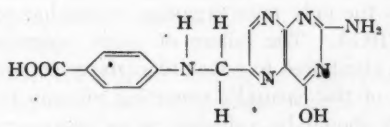
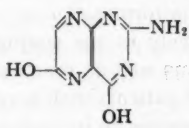
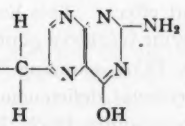
Inasmuch as our previous findings[7] had shown that the SLR factor, which is fully active for *Streptococcus fecalis* and inactive for *Lactobacillus casei*, had low but definite activity for *T. geleii*, it was of importance to test the activity of synthetic pteroic acid, which is fully active for *S. fecalis* and inactive for *L. casei*. Pteroic acid proved to be entirely without activity in concentrations as high as 0.5 microgram per ml., either with or without added glutamic acid. By comparison with the SLR factor,[7] this amount should have been adequate for half-maximum growth of *T.*

*geleii* if the two are identical. No toxicity of the pteroic acid was noted at this level.

Xanthopterine and 2 amino-4 hydroxy-6 methyl-pteridine were without activity, either alone or in combination with PABG. These results are summarized in table 3.

TABLE 3

GROWTH RESPONSE OF *Tetrahymena geleii* W TO VARIOUS COMPOUNDS OF THE "FOLIC ACID" GROUP

| COMPOUND | ½ MAX. ACTIVITY γ ML. |
|---|---|
| PGA  | 0.00064 |
| PTGA (expressed as PGA content) | 0.0005 |
| PHGA (expressed as PGA content) | 0.00032 |
| PABG  | Not active |
| Pteroic Acid  | Not active |
| Xanthopterine  | Not active |
| Methyl Pteridine  | Not active |
| Pteroic Acid + Glutamic Acid | Not active |
| Xanthopterine + PABG | Not active |
| Methyl Pteridine + PABG | Not active |

*Discussion.*—The fact that synthetic pteroic acid fails to give growth at levels at which the SLR factor does, would at first thought appear to indicate their non-identity. An earlier report to this effect has been published.[8] However, when it is recalled that the sample of SLR used is a concentrate from natural sources, rather than a synthetic product, it would appear likely that a slight contamination with PGA was present. This would readily account for the results, since the amount of SLR factor required for half-maximum growth was about 500 times as great as the amount of PGA required. Thus, the presence of 0.02 per cent PGA in the sample would be sufficient to give the results obtained.[7] It may be, therefore, that the SLR factor and pteroic acid are identical.

The results with pteroic acid also indicate that *T. geleii* is unable to form the specific peptide bond which links glutamic acid to the PAB portion of the pteroic acid molecule in the formation of PGA.

Similarly it appears that this ciliate cannot join a pteridine ring to the amino group of either free PAB or of PABG. When xanthopterine was found to be inactive, the possibility still remained that a pteridine with a methyl or hydroxymethyl group in position 6 would exhibit activity. The failure to synthesize PGA from PABG and xanthopterine might have been due to an inability to transmethylate either the PABG or the xanthopterine. Whether or not this transmethylation is possible cannot be decided, since the organism also failed to synthesize PGA from PABG and the methyl-pteridine. It would be of interest to test its ability to utilize *p*-methylimino-benzoyl glutamic acid and xanthopterine, but the former compound was not available.

*Tetrahymena geleii* is the only microörganism which has so far proved capable of utilizing PHGA. The failure of other microörganisms to utilize PHGA has been attributed to a lack of carboxy-peptidase.[5,6] Since by far the larger part of the naturally occurring vitamin is in the conjugated form, *T. geleii* should be valuable as an assay organism. Dr. O. D. Bird is at present investigating the formulation of such an assay procedure. The occurrence of PGA largely in the conjugated form in natural foodstuffs is of great theoretical as well as practical importance. It is this fact which explains the failure of patients with pernicious anemia to recover on a natural diet. Free PGA causes an immediate remission of the symptoms, while PHGA is without effect.[6] This has been attributed to the fact that in such patients the enzyme (carboxy-peptidase) responsible for splitting the conjugated form to free PGA is lacking.[10]

In macrocytic anemias due to nutritional deficiencies the situation is different, since any one of the three compounds, PGA, PTGA, or PHGA, is effective.[11] Whether or not activity increases with the addition of more and more glutamic acid residues as it does in the case of *T. geleii*, remains to be seen, although there seems to be some indication that this may be

so. Totter[11] reports that PTGA gives a better and more lasting response than equal amounts of PGA. On the other hand, Hutchings, *et al.*,[12] report equal activity for PTGA and PGA in producing growth and stimulating hematopoiesis in the chick, but they concluded that biological variability in these experiments makes it difficult to assess the comparative activity.

It is likewise difficult to find an explanation for the greater activity of PHGA. In other microörganisms (e.g., *L. casei*) this compound is practically devoid of activity, indicating a lack of conjugase. It seems evident that other organisms also require at least a part of the vitamin in the free form. In addition it is apparent that at least part of the PGA is stored in the conjugated form. It is known that *T. geleii* stores relatively large amounts of the PGA with which it is supplied.[2] It is therefore possible that stores of PGA as PHGA must first be replenished, while free PGA is utilized in metabolism. Pfiffner[5] makes the statement that PHGA is utilized directly in metabolism, but does not offer experimental support for this statement. That free PGA must be required at least in part is evidenced by the results on pernicious anemia patients. It is also possible that free PGA is required for hematopoiesis and not for other metabolic functions. If it is true that storage of PHGA is compulsory, then, when only free PGA is supplied, storage would be less efficient because synthesis of both glutamic acid and of PHGA are involved. When PHGA is supplied storage proceeds immediately and only a small portion need be hydrolyzed for metabolic needs. This would also explain the intermediate position of PTGA in the scale of potency. Effectiveness of the various compounds appears to increase directly with the number of glutamic acid residues present.

*Summary.*—Vitamin $B_c$ conjugate (pteroylhexaglutamylglutamic acid) is utilized by the ciliate *Tetrahymena geleii* W. On the basis of its pteroylglutamic acid content it is twice as active as the free acid. Fermentation factor (pteroyldiglutamylglutamic acid) is utilized and its activity is intermediate between the other two compounds, on the basis of its pteroylglutamic acid content. Xanthopterine, *p*-aminobenzoyl glutamic acid, pteroic acid and a methyl pteridine proved to be inactive, either alone or in combination.

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[1] *Ann. N. Y. Acad. Sci.*, **48**, 255 (1946).

[2] Kidder, G. W., *Arch. Biochem.*, **9**, 51 (1946).

[3] Kidder, G. W., and Dewey, V. C., *Arch. Biochem.*, **6**, 425 (1945); Kidder, G. W., and Dewey, V. C., *Physiol. Zool.*, **18**, 136 (1945); Kidder, G. W., and Dewey, V. C., *Biol. Bull.*, **89**, 229 (1945); Kidder, G. W., *Ann. N. Y. Acad. Sci.* (in press) (1947).

[4] Pfiffner, J. J., Calkins, D. G., O'Dell, B. L., Bloom, E. S., Brown, R. A., Campbell, C. J., Bird, O. D., *Science*, **102**, 228 (1945).

[5] See review by Pfiffner, J. J., and Hogan, A. G., in *Vitamins and Hormones*, Vol. IV

(1946); and Welch, A. D., Heinle, R. W., Nelson, E. M., and Nelson, H. V., *Ann. N. Y. Acad. Sci.*, **48**, 347 (1946).

[6] Heinle, R. W., and Welch, A. D., *Ann. N. Y. Acad. Sci.*, **48**, 343 (1946).

[7] Kidder, G. W., and Fuller, R. C., *Science*, **104**, 160 (1946).

[8] Kidder, G. W., *Ann. N. Y. Acad. Sci.* (in press) (1947).

[9] Lampen, J. O., and Jones, M. J., *Jour. Biol. Chem.*, **166**, 435 (1946).

[10] Welch, A. D., *et al.*, *Ann. N. Y. Acad. Sci.*, **48**, 347 (1946).

[11] Totter, J. R., *Ibid.*, **48**, 309 (1946).

[12] Hutchings, B. L., Oelson, J. J., and Stokstad, E. L. R., *Jour. Biol. Chem.*, **163**, 447 (1946).

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## *BLOOD GROUPS OF THE RAT (RATTUS NORVEGICUS) AND THEIR INHERITANCE*

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Earlier studies of rat blood were largely exploratory. Rohdenberg (1920) was unable to detect agglutination in mixtures of blood from 50 rats. Lambert (1927) failed to find agglutination in blood mixtures from 46 rats of five different strains. Dr. Hibino, however, working with Dr. Furuhata, an authority on the blood groups of the Japanese, demonstrated isohaemagglutination in the rat, but did not continue the work (personal communication in 1934). Friedberger and Taslokawa (1928) found numerous cases of agglutination between the blood of wild and tame rats in Berlin, and postulated four haemagglutinins with corresponding agglutinogens.

My own studies were begun at the Bussey Institution of Harvard University in 1932, at the suggestion of Dr. W. E. Castle, and were continued subsequently at the University of Maryland.

Blood group differences in animals depend upon the existence of two complementary agencies in blood, an agglutinogen carried in blood cells, and an agglutinin carried in blood plasma. Clumping of the blood cells occurs when the two agencies are brought together. Blood which contains a particular agglutinogen regularly lacks the corresponding agglutinin; otherwise it would clump spontaneously.

Consequently agglutination occurs only when blood from different individuals is mixed, one supplying the agglutinogen, the other the agglutinin.

Animals of a species may be classified in blood groups on the basis of their possession or lack of particular agglutinogens.

In a search for blood groups in a species in which their existence is un-

certain, it is desirable to make combinations of blood from as many unrelated stocks as possible. In the present investigation rats were obtained for study from 15 different laboratory stocks, in addition to wild rats caught at Forest Hills, Mass.

In the course of the investigation, two agglutinogens have been found, one of which resembles the A and B agglutinogens of human blood, the other resembling the M and N agglutinogens of human blood. Because of these resemblances it seems appropriate to designate the newly discovered agglutinogens of the rat A and M respectively.

The agglutinin which acts in conjunction with agglutinogen A to effect blood clumping is found as a natural ingredient of the blood serum of *all rats which do not possess the A agglutinogen*. It may be called agglutinin *a*.

An agglutinin, which will act in conjunction with agglutinogen M to induce blood clumping, does not exist as a natural ingredient of rat blood, at least not in detectable amounts. But it can be artificially produced by injections of blood containing agglutinogen M into animals which lack M. The agglutinin, which may be called *m*, is produced as an antibody to the foreign substance, M. Blood serum containing such an antibody is called an immune serum.

To secure blood for injection or for agglutination tests, the end of the tail may be snipped off, the animal being first etherized. But the yield by this method is small, usually 2 cc. or less, and the product frequently contaminated.

A better method is to bleed from the heart, as described by Burhoe, 1940. The yield is larger and more likely to be sterile.

To obtain serum, the blood is collected in 6 cc. agglutination tubes and allowed to clot. The clot is broken up with a clean probe or small twisted wire, and the material centrifuged.

In preparation for testing the agglutinating properties of a rat's blood corpuscles, a few drops of freshly drawn blood are put in a mixture of 1% sodium citrate in physiological salt solution.

Injections for the production of an immune serum may be made either subcutaneously or into the body cavity. An injection of from 1 cc. to 5 cc. of blood should result in producing immune agglutinin in about five days. The immunity, however, gradually disappears thereafter and is entirely gone within two months, unless the injection is repeated.

The presence of agglutinin *a* in a rat does not preclude the development of agglutinin *m* along with it. Thus a rat prossessing *a* may be made to develop *m* also, if agglutinogen M is injected into it, resulting in bivalent serum, $a + m$.

In the initial experiments mixing of blood from different laboratory stocks gave negative results (no clumping) as a like procedure had in the case of many earlier investigators, but finally a stock of red-eyed yellow

rats supplied by Dr. H. W. Feldman of the University of Michigan, showed clumping of blood cells introduced into its serum from all other races tested. This result indicated that the yellow race contained in its serum an agglutinin which was effective in the clumping, but was an exclusive possession of that particular race.

This race was the original source of agglutinin *a*. Its serum was used in diagnosing the blood constitution (presence or absence of agglutinogen A) of other laboratory stocks and of captured wild rats. In fact all animals so tested in the initial experiments were found to have A. But when crosses were made between the peculiar red-eyed yellow race and other stocks, there appeared in the $F_2$ generation an abundance of animals (recessives) which lacked the A agglutinogen and so naturally possessed the *a* agglutinin.

The existence of a second agglutinogen was demonstrated by the method, originally devised by Landsteiner, of producing immune sera by reciprocal or multiple exchanges of blood between individuals. In exploratory studies of the rat being undertaken in this case, the method is particularly effective when races differing as widely as possible are used. The immune serum *m* was produced thus. Blood was taken from a selected individual of each of eleven different laboratory stocks. Two cc. of blood were drawn from each donor, centrifuged to separate the blood cells, which were then washed in saline, pooled and injected intraperitoneally at semi-weekly intervals into an individual of each of the eleven races which had furnished the blood cells. Tests for the presence of an agglutinin were thereafter made every two weeks, using serum of each injected animal, into which pooled blood cells of the donors were introduced.

After six weeks of injections the immune agglutinin was detected in the sera of certain of the injected animals, its presence being revealed by clumping of the introduced blood cells. For example, serum of the injected individual of family D was found to clump cells of families E, H, and J. Further tests made with the newly produced serum, showed that of the 16 families included in the study, 9 laboratory stocks consisted wholly or in part of individuals carrying the M agglutinogen, while the wild rats tested all were carriers of it. Four different albino stocks, including two Wistar albino strains, and a black strain supplied by Dr. Feldman were found to lack the M agglutinogen but to carry A. Only one family, the red-eyed yellow family supplied by Feldman, carried neither agglutinogen.

Demonstration of the existence of two different agglutinogens in the rat make it possible to classify individuals in four blood groups, viz., (1) those which carry both A and M (group AM), (2) those which carry A but not M (group A), (3) those which carry M but not A (group M) and (4) those which carry neither A nor M (group O).

The results of crosses made between individuals of the four blood groups

are summarized in table 1. They show the character of each individual tested with diagnostic sera in 508 litters of rats aggregating 3203 individuals. The crosses show consistently that the agglutinogens are inherited as dominant characters and assort independently, which means that their genes are carried in different chromosome pairs.

TABLE 1

DATA ON THE INHERITANCE OF THE TWO BLOOD GROUP GENES, A AND M

| CROSS NO. | GENOTYPE OF PARENTS | NO. OF LITTERS | BLOOD GROUPS OF YOUNG | | | | TOTAL YOUNG |
|---|---|---|---|---|---|---|---|
| | | | AM | A | M | O | |
| 1 | O × O | 9 | ... | ... | ... | 53 | 53 |
| 2 | AAMM × AAMM | 10 | 61 | ... | ... | ... | 61 |
| 3 | AA × AA | 19 | ... | 93 | ... | ... | 93 |
| 4 | AAMM × O | 16 | 92 | ... | ... | ... | 92 |
| 5 | AM × AM | 23 | 88 | 32 | 33 | 10 | 163 |
| 6 | AM × O | 50 | 102 | 85 | 74 | 89 | 350 |
| 7 | AMM × O | 4 | 15 | ... | 13 | ... | 28 |
| 8 | A × M | 14 | 29 | 21 | 16 | 21 | 87 |
| 9 | AAM × O | 6 | 28 | 25 | ... | ... | 53 |
| 10 | M × M | 16 | ... | ... | 78 | 32 | 110 |
| 11[a] | AA × O | 8 | ... | 50 | ... | ... | 50 |
| 11[b] | $F_2$, A × A | 24 | ... | 100 | ... | 42 | 142 |
| 11[c] | BC, A × O | 90 | ... | 233 | ... | 262 | 545 |
| 12[a] | MM × O | 11 | ... | ... | 61 | ... | 61 |
| 12[b] | $F_2$, M × M | 43 | ... | ... | 177 | 71 | 248 |
| 12[c] | BC, M × O | 89 | ... | ... | 282 | 293 | 575 |
| 13[a] | AA × MM | 2 | 7 | ... | ... | ... | 7 |
| 13[b] | $F_2$, AM × AM | 19 | 65 | 24 | 23 | 7 | 119 |
| 14[a] | AAMM × O | 14 | 80 | ... | ... | ... | 80 |
| 14[b] | BC, AM ($F_1$) × O | 41 | 77 | 70 | 64 | 75 | 286 |
| | Totals | 508 | ... | ... | ... | ... | 3203 |

From an examination of table 1 the following conclusions may be drawn:

1. Group O individuals are double recessives, carry neither dominant gene, and breed true (cross 1).

2. Group A individuals may be either homozygous (AA) or heterozygous (A). When homozygous they produce only group A progeny in matings with group O individuals (cross 11[a]). When heterozygous they produce both A and O individuals in a 1:1 ratio (cross 11[c]).

3. Group M individuals also may be either homozygous (MM) or heterozygous (M). When homozygous they produce only group M progeny in matings with group O individuals (cross 12[a]). When heterozygous they produce both M and O progeny in a 1:1 ratio (cross 12[c]).

4. Group AM individuals carry both dominant genes (A and M) but may be either homozygous for both, heterozygous for one only or heterozygous for both. The four possible varieties of genetic constitution of group AM individuals are shown in the following crosses: AAMM in

crosses 4 and 14[a]; AAM in cross 9; AMM in cross 7; AM in crosses 6 and 14[b] (in which a 1:1:1:1 ratio is approximated).

5. When double heterozygotes (AM) are mated together, a 9:3:3:1 ratio is obtained (crosses 5 and 13[b]).

We may conclude that the inheritance of the two agglutinogens, A and M, is in every respect typically mendelian, that the two dominants assort independently and so are undoubtedly carried in different chromosome pairs.

*Linkage Studies*—In order to identify, if possible, the chromosome pairs in which the genes for the agglutinogens A and M are located, tests have been made for linkage with eight different mutant genes of the rat, each of which is thought to be located in a different chromosome pair. The results of these tests are summarized in tables 2 and 3.

TABLE 2

DATA FROM TESTS FOR LINKAGE BETWEEN THE GENE FOR AGGLUTINOGEN A AND EIGHT OTHER MUTANT GENES OF THE RAT. D MEANS THE DOMINANT ALLELE OF THE CHARACTER UNDER INVESTIGATION, O MEANS DOUBLE RECESSIVE

| MUTANT GENE AND NATURE OF CROSS | CLASSES OF YOUNG DA | D | A | O | TOTAL YOUNG | CROSS-OVERS | NON-CROSS-OVERS | DEV/PE |
|---|---|---|---|---|---|---|---|---|
| Agouti, R | *23* | 21 | 25 | *22* | 91 | 45 | 46 | 0.1 |
| Kinky, R | *25* | 27 | 21 | *22* | 95 | 47 | 48 | 1.5 |
| Red-eye, R | *14* | 11 | 16 | *17* | 58 | 31 | 27 | 0.8 |
| Curly, C | 14 | *14* | *15* | 16 | 59 | 29 | 30 | 0.1 |
| Curly$_2$, C | 22 | *20* | *20* | 22 | 84 | 40 | 44 | 0.6 |
| Blue, C | 19 | *20* | *15* | 21 | 75 | 35 | 40 | 0.9 |
| Hairless, C | 39 | *30* | *39* | 38 | 146 | 69 | 77 | 0.9 |
| Hooded, $F_2$, R | 97 | *30* | *30* | 9 | 166 | 60 (expected 62) | | 0.5 |

Tests for linkage of A are shown in table 2. Crosses were first made to produce individuals doubly heterozygous for gene A and one of the mutant genes under investigation. Then the double heterozygote was crossed to the appropriate double recessive, if such was available. In table 2, the first seven crosses listed were of this nature, a double heterozygote being mated to a double recessive. If the original cross was *repulsional*, this is indicated by R in the table, first three and last entry. If the cross was coupling in character, this is indicated by C, four entries. The classes of young which are *crossover* (recombinations) are italicized.

In the case of the hooded gene, no double recessive was available for crossing with the double heterozygote. Consequently an $F_2$ population was produced. In this case the two middle classes of young, numbering 30 each, are recombination classes which would involve, in the production of every individual, either one or two crossover gametes. If there is no linkage (repulsion) between A and the gene for hooded, we should expect the middle classes to contain ten-sixteenths of the population of 166 individuals,

i.e., 62. Actually they contain 60, a deviation without statistical significance.

If there were linkage, we should expect a significant diminution in these two classes from the calculated total, 62. Since it does not occur, it is fair to conclude that existence of linkage is highly improbable in this case.

The seven other linkage tests recorded in table 2 were made by the preferable method of crossing an $F_1$ individual to double recessive mates. In each instance an equality of crossover and non-crossover individuals is expected, and from this expectation no significant deviation is observed, as shown in the last column of the table.

TABLE 3

DATA FROM TESTS FOR LINKAGE BETWEEN THE GENE FOR AGGLUTINOGEN M AND EIGHT OTHER MUTANT GENES OF THE RAT. D MEANS THE DOMINANT ALLELE OF THE CHARACTER UNDER INVESTIGATION, O MEANS DOUBLE RECESSIVE

| MUTANT GENE AND NATURE OF CROSS | CLASSES OF YOUNG | | | | TOTAL YOUNG | CROSS-OVERS | NON-CROSS-OVERS | DEV/PE |
|---|---|---|---|---|---|---|---|---|
| | DM | D | M | O | | | | |
| Agouti, C | 34 | *35* | *42* | 31 | 142 | 77 | 65 | 1.5 |
| Curly, C | 14 | *14* | *14* | 17 | 59 | 28 | 31 | 0.6 |
| Curly$_2$, C | 18 | *26* | *20* | 22 | 86 | 46 | 40 | 0.9 |
| Hairless, R | *19* | 17 | 11 | *12* | 59 | 31 | 28 | 0.4 |
| Hooded, R | 34 | *27* | *35* | 28 | 124 | 62 | 62 | 0 |
| Blue, $F_2$, C | 50 | 17 | 19 | *5* | 91 | 5 (expected 5.7) | | 0.4 |
| Kinky, $F_2$, R | 81 | *33* | *15* | 6 | 135 | 48 (expected 50.6) | | 0.7 |
| Red-eye, $F_2$, R | 67 | *23* | *18* | 8 | 116 | 41 (expected 43.5) | | 0.7 |

In table 3 tests for linkage of the same eight mutant genes with M are recorded. Here are shown the results of three original coupling crosses subsequently back-crossed to the double recessive (first three entries). There follow two repulsion crosses similarly back-crossed to the double recessive. Finally listed are three $F_2$ populations from crosses, one of which involved the coupling relationship, and the last two the repulsion relationship. No indication of linkage is found in the five back-cross experiments.

The $F_2$ tests for kinky and red-eye (last two entries) are similar in character to the hooded test in table 2 already discussed and have a similar outcome. No significant deviation is shown from the numbers expected in the two middle (exclusively crossover) classes, if no linkage exists.

The $F_2$ test for blue was based on a coupling cross. Here the double recessive class (4th column) could arise only from recombination (crossover) gametes. Its frequency is 5, where the maximum expectancy, if no linkage exists, is 5.7, a non-significant difference.

We may conclude that the experiments summarized in tables 2 and 3 give no indication of linkage between the genes for agglutinogens A and M and genes serving as genetic markers of eight pairs of autosomes of the rat.

Since we must conclude that the genes for A and M do not lie in any of the eight chromosome pairs tagged by the mutant genes listed in tables 2 and 3, it follows that they will constitute marker genes, respectively, for a 9th and 10th autosomal pair. Dr. Castle informs me that in recent years the number of known mutant genes has been increased to 22. It is quite possible that some of the newly discovered mutant genes, not listed in tables 2 and 3, may actually lie in chromosomes for which agglutinogens A and M now serve as markers. To ascertain this, further linkage studies are needed.

*Summary.*—1. In an attempt to discover blood groups in the rat, 15 different laboratory stocks and a collection of wild rats have been studied.

2. The wild rats and ten of the laboratory stocks were found to be carriers of two different agglutinogens, A and M. Four of the laboratory stocks carried A only, and one stock carried neither A nor M.

3. Rats which lack A, either by original mutation or by genetic recombination following a cross with a race lacking A, have as a natural ingredient of their serum an agglutinin which will cause clumping of the blood corpuscles of a rat having agglutinogen A. This natural agglutinin may be called agglutinin *a*.

4. Agglutinogen M is capable of demonstration only by immune serum created by injection of blood containing M into rats which lack it. Such an artificially induced agglutinin may be called *m*. It causes agglutination of blood cells containing M, when they are introduced into it.

5. On the basis of the presence in or absence from individual rats of the agglutinogens A and M, rats may be classified in four blood groups, AM, A, M and O. Wild rats (so far as studied) and many laboratory stocks (Long-Evans, at least in part) are AM. Most stocks of albino rats (including Wistar stock albinos) are A, as are also some colored stocks. One stock only has been found to be O. An M group has been obtained as and $F_2$ recombination class, following a cross between AM and O individuals.

6. Presence of Agglutinogen A or agglutinin *a* in a rat does not interfere with the development also in it of agglutinin *m*, upon injection of M cells into said rat. A bivalent test serum results, *a* and *m*.

7. Agglutinogens A and M are inherited as simple dominant characters, and may occur either as homozygotes or as heterozygotes, together or apart. They segregate and recombine independently, and behave in every respect as typical autosomal characters.

8. No indications were found of linkage of either A or M with eight mutant genes believed to be borne in as many different chromosome pairs.

* Based in part on a thesis for the degree of Ph.D. presented to the Biological Faculty of Harvard University in 1937.

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## *THE DOMESTICATION OF THE RAT*

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The Norway rat (Rattus norvegicus) is a comparatively recent immigrant to Europe and America and at the present time is reproducing in enormous numbers both in a wild state and under domestication. A comparative study of its behavior in the two contrasted states is thus made easy and should throw light on what takes place in a species of mammal when it is brought into captivity and its breeding is controlled by man.

The Norway rat entered western Europe by way of the Norwegian peninsula in the first half of the Eighteenth Century and bears a specific name indicating the route by which it arrived. Its ecological predecessor was the black rat (Rattus rattus) the rat which spread plague in London in 1664–1665. This species was soon afterward replaced in Western Europe by its mortal enemy the newly introduced Norway rat, which promptly made its way on ships to the New World, where the black rat had preceded it but was, as in Europe, promptly supplanted by the Norway rat except in out-lying districts such as northern New Hampshire where Dr. C. C. Little secured for me live examples of Rattus rattus about 1910, and on which Dr. H. W. Feldman made genetic studies. One interesting result of these studies was the demonstration that crosses between the two species, R. rattus and R. norvegicus, are very difficult to obtain: embryos never coming to term alive. So it is easy to see why hybrids do not occur in nature.

At sometime after the introduction of the Norway rat into Western Europe, it is probable that albino mutants made their appearance in the

wild population, as they do in the case of most wild mammals. Albino individuals were probably captured and tamed because of their attractive and distinctive appearance and thus the tame white rat became the earliest domesticated type of rat. Similarly a black (non-agouti) mutation made its appearance, and also a piebald (hooded) mutant appeared, perhaps originally gray-and-white in color, but if mated to black individuals resulting in the production of the double recessive black hooded type. Then if the black hooded type was crossed with albinos, triple recessives would result, albinos homozygous for black and piebald (*cc aa hh*). Keeler has shown that black individuals are in temperament gentler than grays of like ancestry and it seems probable that this made easier the process of taming the mutant strains, since the gentler individuals would be more amenable to handling and confinement and would thus come to predominate in the domesticated race.

At any rate we know that when the first recorded breeding experiments with white rats were undertaken by Crampe about 1880, an albino female which he mated to a wild gray male transmitted as recessives to her gray $F_1$ offspring the three mutant genes, *c* (albino), *a* (non-agouti), and *h* (hooded). Although Crampe's experiments were made in the pre-Mendelian period, his records as analyzed by Doncaster (1906) fully support this interpretation.

It is clear also that when white rats of European origin were brought to America (by Dr. H. H. Donaldson and others) and were made the foundation of the Wistar Institute race of albinos, these were homozygous for the same three mutant genes (*cc aa hh*) as were Crampe's albino. Albino rats which Dr. Donaldson kindly supplied to me about 1903 were of this genetic constitution.

Dr. Donaldson in 1906 transferred his colony of albino rats from the University of Chicago to the Wistar Institute. Here Dr. Helen Dean King in 1908 became associated with him in a comparative study of the albino rat and its wild gray ancestor. She had the happy thought that it would be interesting to re-enact the domestication of the rat under controlled conditions and thus to observe just what occurs in the process. In this she had the coöperation and support of Dr. Donaldson, with consequences of the highest importance to the science of genetics. In the spring of 1919 Dr. King began rearing in captivity the progeny of wild rats captured in the vicinity of Philadelphia. In 1929 she reported on the life processes observed in the first 10 generations of captive gray rats, Dr. Donaldson at the same time reporting on size of body and organs of the rats. Ten years later, after Dr. Donaldson's death, Dr. King made a further report on life processes as observed in 26 generations of captive rats.

Donaldson in 1929 had summarized as follows the initial differences

between wild gray rats and albinos. "The wild Norway are more excitable and much more savage. They gnaw their cages. The body weight is less for a given body length, hence it is a slighter animal. The skeleton is relatively heavier, also the suprarenals (both sexes) and the testes and ovaries. The thyroid is of like weight, but the hypophysis distinctly lighter in both sexes. On the other hand, the brain and the spinal cord are both heavier than in the Albino."

After ten generations in captivity Donaldson finds that in captive grays there has been an increase in body weight in relation to body length, i.e., the body has become less slender, more like the albino in conformation. The hypophysis has increased slightly in weight. No change has occurred in the weight of the gonads. Decrease in weight is shown by brain, thyroid and suprarenals. But brain, suprarenals, gonads and bones are still heavier than in the Albino race. "Ten generations of captivity have, by no means, he says, served to give the captive Grays the organ constitution of the Albino." It would seem, accordingly, that a changed and controlled environment had effected little racial change in the course of ten generations.

As regards the changes observed in life processes during 25 generations in captivity Dr. King (1939) notes a gradual increase in the "rate and extent of body growth," i.e., in general body size. "At the last generation growth acceleration (more rapid growth during the adolescent period) was nearly equal to that found in stock albino rats that have been under domestication for a long period of time. At the twenty-fifth generation adult rats of both sexes were, on the average, about 20 per cent heavier than individuals of the first generation."

"Rats attaining an adult weight much above the average for all individuals of like sex in the same generation group appeared in increasing numbers as the generations advanced." The weight increase is ascribed tentatively to genetic mutation rather than to a direct effect of a changed environment.

At the twenty-fifth generation the average length of the reproductive period was nearly 8 months longer than for the first generation. This extension resulted from the earlier breeding of the rats and the persistence of reproduction to a more advanced age.

Fertility of the rats, as measured by litter production increased steadily reaching its maximum at the nineteenth generation where females produced an average of 10.18 litters each, as contrasted to an average of 3.5 litters each for generation one. No significant change in the size of individual litters was observed. Litter size continued at an average of 6.1 throughout generations 2–26. Variability in body size decreased, i.e., the race became more uniform in body size.

On the whole it would seem that in the experiments of Dr. King genetic

differences present in the foundation animals or mutational changes occurring in their descendants will account adequately for the changes observed.

Those changes are (1) accelerated growth rate resulting in increased body size; (2) decreased "nervous tension" resulting in tamableness when the animals were handled frequently in early life; (3) mutations in color or structure of the hair.

In the course of Dr. King's experiments with captive wild gray rats, she observed the new occurrence among them of four mutations previously known, *c*, *a*, *h* and $c^d$ of table 1. Of these *h* was already present as a re-

TABLE 1

MUTATIONS OF THE RAT

| DESIGNATION | GENETIC SYMBOL AND LINKAGE GROUP | NATURE | TIME AND PLACE OF ORIGIN | RECORDED BY | NATURE OF POPULATION IN WHICH IT APPEARED |
|---|---|---|---|---|---|
| 1. Albino | *c* I | Absence of pigment from coat and eyes | 17th or 18th Centuries in Western Europe | H. Crampe, 1885 | Wild |
| 2. Non-agouti | *a* | Absence of wild coat pattern, uniform black | 17th or 18th Centuries in Western Europe | H. Crampe, 1885 | Wild |
| 3. Hooded | *h* I | White except head and back stripe | 17th or 18th Centuries in Western Europe | H. Crampe, 1885 | Wild |
| 4. Pink-eyed yellow | *p* I | Coat yellow, eyes pink | 1907, England | Castle | Wild |
| 5. Red-eyed yellow | *r* I | Coat yellow, eyes red | 1907, England | Castle | Wild |
| 6. Curly | *Cu* II | Hairs of coat and vibrissae curved | 1920–1930 Wistar Institute | Helen Dean King | Captive wild |
| 7. Brown | *b* II | Black pigment of coat and eyes replaced by brown | 1920–1930 Wistar Institute | Helen Dean King | Captive wild |
| 8. Stub | *st* IV | Short stubby tail | 1939 Wistar Institute | Helen Dean King | Captive wild |
| 9 Ruby-eyed dilute | $c^d$ I | Allele of albino gene, *c*, pigmentation diminished | 1918 in wild rats in Phila.; later in captive grays, 1920–1930 | Whiting and King 1918, King 1939 | Captive wild |

| | | | | | | |
|---|---|---|---|---|---|---|
| 10. | Curly$_2$ | *Cu*$_2$ | Coat hairs and vibrissae strongly curved | Davis, Calif., 1935 | Blunn and Gregory | Long-Evans captive gray stock |
| 11. | Kinky | *k* IV | Coat hairs and vibrissae strongly curved | Ann Arbor, Michigan, 1935 | H. W. Feldman | Domesticated strain |
| 12. | Lethal | *l* I | Skeleton imperfect | England 1939 | H. Grüneberg | Domesticated strain |
| 13. | Blue | *d* | Black pigment diluted (clumped) to yield a blue | University of Illinois, 1929 | E. Roberts | Domesticated strain |
| 14. | Hairless | *hr* III | Hair lost at about 4 weeks of age | University of Illinois, 1940 | E. Roberts | Domesticated strain |
| 15. | Wobbly | *wo* III | Ataxic locomotion | Univ. of Iowa, 1941 | Castle, King, and Daniels | Domesticated strain |
| 16. | Waltzing | *w* I | Runs in circles | Wistar Institute, 1936 | Helen Dean King | Domesticated strain |
| 17. | Incisorless | *in* II | Incisors lacking | Squibb Labs., New Brunswick, N. J., 1941 | R. O. Greep | Domesticated strain |
| 18. | Anemia | *an* II | Young anemic at birth, lack of red blood cells | Cornell Univ., 1939 | Smith and Bogart | Domesticated strain |
| 19. | Cataract | *Ca* | Opaque lens visible in unpigmented eyes, pink-eyed or albino | Cornell Univ., 1943 | Smith and Barrentine | Domesticated strain |
| 20. | Jaundice | *j* | Skin and hair yellow at birth and later | Univ. of Toronto, 1938 | C. H. Gunn | Domesticated strain |
| 21. | Shaggy | *Sh* II | Hair and vibrissae curved, closely linked to curly | Wistar Institute, 1946 | Helen Dean King | Domesticated strain |

(*Table continued on following page*)

| | | | | | | |
|---|---|---|---|---|---|---|
| 22. | Silver | *s* | Black coat interspersed with white hairs | Wistar Institute, 1939 | Helen Dean King | Domesticated strain |
| 23. | Fawn | *f* | Dilutes black to tawny, blue to fawn | Wistar Institute, 1946 | Helen Dean King | Domesticated strain |

cessive in one animal of the foundation wild stock. Three previously unknown mutations of the rat made their appearance in the captive stock, curly in generation 17, brown in generation 22, and stub seven generations later.

Castle had reported in 1907 the occurrence in wild rats in England of mutations *p* and *r*. One of these, *r*, made its appearance independently in Wistar Institute stock (not captive gray) as reported by King, 1923.

Meanwhile several other mutations had been observed in domestic laboratory stocks. Roberts in 1924 reported the occurrence of hairless (*hr*), and in 1929 of blue dilutin (*d*). Wilder (1932) observed an independent occurrence of the hairless mutation, and Feldman demonstrated the identity of the two. Gregory and Blunn in 1935 reported the occurrence of a second dominant curly mutation which they designated $Curly_2$, and showed to be distinct from Curly, the two being independent in inheritance, and so obviously borne in different chromosome pairs. In the same year (1935) Feldman discovered a recessive form of curly hair which he named kinky (*k*).

In 1936 King reported the discovery of a recessive gene for waltzing (*w*) in Wistar albino rats, mutants from captive grays. In (1937) Daniels discovered a recessive gene for wobbly, which was described and its linkage relations canvassed by Castle, King, and Daniels. In 1938 Gunn described a recessive gene for jaundice. In 1939 Smith and Bogart reported the discovery of a recessive lethal, anemia (*an*); and Grüneberg reported on a different lethal (*l*) resulting in an abnormal skeleton. This he showed to be carried in the albino chromosome. In 1941 Greep reported the discovery of incisorlers (*in*). In 1943 Smith and Barrentine reported the discovery of a new dominant mutation cataract (*Ca*). King has also discovered three other mutations on which as yet no publication has been made. They are silver, a recessive; fawn, also recessive; and shaggy, a dominant resembling the curly mutations.

Burhoe, studying the blood groups of the rat, has demonstrated the existence of two dominant agglutinogen genes, *Ag* and *M*.

One striking fact concerning the mutations of the rat is that they may occur again independently of an original and earlier occurrence. King has demonstrated this in her own studies for *c*, *a*, *h* and *r*, also for $Cu_2$ observed

as occurring independently in New Haven, Conn., by Whitney. The blue mutation (*d*) originally observed by Roberts in 1929 was shown to have occurred independently later in New York (Curtis and Dunning, 1940). Curly, as well as Curly$_2$, has made a second independent appearance, at Madison, Wis., (personal communication from Dr. A. B. Chapman).

Linkage studies made to discover what genes are carried in a common chromosome pair have been made by Roberts and Quisenberry (1936), by Burhoe and by Feldman, but all their findings were negative. The first positive finding was made by Castle and Wright, who showed that the two genes for yellow coat, *p* and *r* are linked with each other. Later it was found by Castle, Dunn and Wachter that they lie in the same chromosome pair as the albino gene. Castle and King found that the gene for waltzing also lies in the albino chromosome, and Grüneberg added a fifth gene, a lethal *l* to this first linkage group. A second linkage group was found by King and Castle to include curly and brown, to which later were added the genes shaggy, anemia and incisorless. A third linkage group was found by Castle, King and Daniels to include the genes wobbly and hairless. A fourth group includes genes kinky and stub, as demonstrated by Castle and King. Conventional linkage maps may be expressed as follows:

| | | | | | |
|---|---|---|---|---|---|
| I | *p* | *r* | *c* | *l* | *w* |
| | 0 | 20.5 | 21 | 24.3 | 66.3 |
| II | *Cu* | *Sh* | *an* | *in* | *b* |
| | 0 | 0.5 | 10.3 | 24 | 45 |
| III | *wo* | | | | *hr* |
| | 0 | | | | 40.3 |
| IV | *k* | | | | *st* |
| | 0 | | | | 34.1 |

For the following genes no linkage has as yet been found, though the investigation is far from complete: *a*, *Ag*, *Ca*, *Cu*$_2$, *d*, *h*, *j* and *M*. If all of these should be shown to be independent of the established linkage groups we should have genetic markers for 12 of the 20 autosomal chromosome pairs, no sex-linked gene having as yet been discovered.

By way of summary we may say that the earliest attempts at domestication of the Norway rat followed the discovery in wild populations of conspicuous mutants, albinos, non-agouti blacks and piebalds. These were captured, and intercrossed, resulting in the formation of a race of albinos homozygous for the three mutant genes *c*, *a* and *h*. Such is the genetic constitution of the ordinary laboratory white rat.

Unconscious selection was probably made of the more gentle and tamable individuals for propagation, which also favored increased productiveness in captivity.

Experimental re-enactment of domestication by taking wild gray rats into captivity has resulted in (1) increased body size, (2) decreased savageness (inclination to bite and attempt to escape) and (3) increased fertility.

These may be regarded as consequences of mutations affecting behavior either directly or by way of endocrine changes, rather than as direct effects of a changed environment. At the same time mutations have been observed to occur which affect the structure of the hair or its pigmentation, or the central nervous system (waltzing, wobbly), the eyes or the skeleton (stub tail, lethal *l*). These are not to be regarded as consequences of domestication but purely as sports, spontaneous and without assignable causation.

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*Ag* is used as the symbol of a gene for agglutinogene A of Burhoe (published herewith) to avoid confusion with *A*, agouti, dominant allele of *a*.

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## *AN EXPRESSION OF HOPF'S INVARIANT* AS AN INTEGRAL*

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1. Let $S^2 \subset R^3$ and $S^3 \subset R^4$ be spheres in the sense of Euclidean geometry, $S^2$ having unit radius, and let $f: S^3 \to S^2$ be a twice differentiable map. Let $x^1, x^2, x^3$ be local coördinates for $S^3$, let $\lambda, \mu$ be local coördinates for $S^2$ and let $\sigma(\lambda, \mu)$ be the area density on $S^2$. Let

$$u_{ij} = \frac{1}{4\pi}\,\sigma(\lambda, \mu)\,\frac{\partial(\lambda, \mu)}{\partial(x^i, x^j)} \quad (i, j = 1, 2\ 3), \tag{1.1}$$

where $\lambda, \mu$, in (1.1) stand for the functions $\lambda(x^1, x^2, x^3)$, $\mu(x^1, x^2, x^3)$, by means of which $f$ is expressed locally. Then $u_{ij}$ are the components of an alternating tensor in $S^3$. It may be verified that the divergence of this tensor vanishes. That is to say

$$\partial u_{23}/\partial x^1 + \partial u_{31}/\partial x^2 + \partial u_{12}/\partial x^3 = 0.$$

Hence, by de Rham's theorem[1] there is a covariant vector-field $(v_1, v_2, v_3)$, defined over the whole of $S^3$, such that

$$\partial v_i/\partial x^j - \partial v_j/\partial x^i = u_{ij}. \tag{1.2}$$

The main object of this note is to prove that

$$\frac{1}{4\pi}\int\int\int_{S^3}\sigma\begin{vmatrix} v_1 & v_2 & v_3 \\ \frac{\partial\lambda}{\partial x^1} & \frac{\partial\lambda}{\partial x^2} & \frac{\partial\lambda}{\partial x^3} \\ \frac{\partial\mu}{\partial x^1} & \frac{\partial\mu}{\partial x^2} & \frac{\partial\mu}{\partial x^3} \end{vmatrix} dx^1dx^2dx^3 = \gamma, \qquad (1.3)$$

where $\gamma$ is the Hopf invariant of the map $f$. Notice that this integral can also be expressed in the form

$$\int_{S^3}\omega_1\square\omega_2 = \gamma, \qquad (1.4)$$

where $\omega_1$ and $\omega_2$ are the forms whose coefficients are $v_i$ and $u_{ij}$, and $\square$ denotes Grassmann multiplication.

Before starting the proof we re-state the theorem in topological terms. Let $w^2$ be the basic co-cycle on $S^2$, defined as a function of singular 2-simplexes, and let $u^2 = f_*w^2$, where $f_*$ stands for the map of co-chains, which is dual to $f$. Since $\delta u^2 = 0$, because $\delta w^2 = 0$, and since the second co-homology group of $S^3$ is trivial, there is a co-chain, $v^1$, such that $\delta v^1 = u^2$. Our theorem is equivalent to the statement

$$v^1 \smile u^2 = \gamma c^3,$$

where $c^3$ is the basic co-cycle on $S^3$. This form of the result has been obtained independently by N. Steenrod, as an application of a process, which he has developed but has not yet published.

2. We shall prove (1.3) with the help of a theorem concerning 3-dimensional manifolds, which are fibred in the original sense of H. Seifert.[2] Let $M^3$ be such a manifold, which we assume to be orientable and closed. Let $f: M^3 \to M^2$ be a fibre mapping of $M^3$ on an orientable, 2-dimensional manifold $M^2$. Let $M^3$, $M^2$, the map $f$ and the fibres be twice differentiable. Let each fibre be oriented so as to have a positive intersection with a transverse 2-cell, which takes its orientation from a given orientation of $M^3$. We recall Seifert's definition of a *fibre neighborhood*, $T$, of a given fibre $H$ (p. 150), and the number $n$, which is the degree of the map $f|E^2$ in the point $f(H)$, where $E^2$ is a cross section of $T$. We shall call $n$ the *order* of the fibre $H$, and $H$ will be described as a *simple* or a *multiple* fibre, according as $n = 1$ or $n > 1$. In either case we may take $T = f^{-1}(E_1^2)$, where $E_1^2 \subset M^2$ is any sufficiently small 2-element, of which $f(H)$ is an inner point.

Let $x^1$, $x^2$, $x^3$ be local coördinates for $M^3$, let $\lambda$, $\mu$ be local coördinates for $M^2$ and let[3] $\sigma(\lambda, \mu)$ be an area density for $M^2$, which may be given abstractly or defined in terms of a Riemannian metric. Let $\alpha$ be the reciprocal of the total area of $M^2$, which is compact since $M^3$ is compact, and

let $u_{ij}$ be defined by (1.1), with $1/4\pi$ replaced by $\alpha$. We assume the existence of a covariant vector-field $v_i$, defined over the whole of $M^3$ and satisfying (1.2). As in Section 1 we write, using the summation convention,

$$(a)\quad \omega_2 = \alpha\sigma(\lambda, \mu)(d\lambda\delta\mu - \delta\lambda d\mu) = u_{ij}dx^i\delta x^j, \qquad (2.1)$$
$$(b)\quad \omega_1 = v_i dx^i.$$

I say that

$$\int_H \omega_1 = \int_{H'} \omega_1 = \gamma, \text{ say}, \qquad (2.2)$$

where $H$ and $H'$ are any two simple fibres and the integrals are both taken in the positive sense and $\gamma$ is thus defined. For $d\lambda = d\mu = 0$ along a fibre whence $\int \omega_2 = 0$ over any surface which is generated by fibres. Let $\Delta \subset M^2$ be a smooth, non-singular arc joining $f(H)$ to $f(H')$ and not containing the image of any multiple fibre. Then $\Sigma = f^{-1}(s)$ is generated by fibres and is obviously bounded by $\pm(H - H')$. Therefore

$$\int_H \omega_1 - \int_{H'} \omega_1 = \pm \int_\Sigma \omega_2 = 0,$$

which establishes (2.2).

3. Let $D$ denote the determinant in (1.3). The theorem referred to in Section 2 is that

$$\alpha \int\int_{M^3}\int \sigma D dx^1 dx^2 dx^3 = \gamma, \qquad (3.1)$$

where $\gamma$ is defined by (2.2). Let $H$ be a simple fibre and $T$ a fibre neighborhood of $H$. We may represent $T$ as the topological product $E^2 \times H$ where $E^2$ is a cross-section of $T$, which is mapped topologically on $f(E^2)$, with non-degenerate Jacobian. We assume that $f(E^2)$ is contained in the domain of a local coördinate system, $(\lambda, \mu)$, for $M^2$, and use the same coördinates for $p \in E^2$ as for $f(p)$. Thus $T$ may be referred to coördinates $(\lambda, \mu, \theta)$, such that $f(\lambda, \mu, \theta) = (\lambda, \mu)$ and $\theta \in \langle 0, 2\pi \rangle$ is a periodic coordinate for $H$. Let $\omega_1 = w_\lambda d\lambda + w_\mu d\mu + w_\theta d\theta$ and let $H(\lambda, \mu) = f^{-1}(\lambda, \mu)$. Then $D = w_\theta(\lambda, \mu, \theta)$ and

$$\gamma = \int_{H(\lambda, \mu)} \omega_1 = \int_0^{2\pi} w_\theta(\lambda, \mu, \theta) d\theta.$$

Hence the integral in (3.1), extended over $T$, becomes

$$\begin{aligned}
&\alpha \int\int_T \int \sigma(\lambda, \mu) w_\theta(\lambda, \mu, \theta) d\lambda d\mu d\theta \\
&= \alpha \int\int_{E^2} \left\{ \sigma(\lambda, \mu) \int_0^{2\pi} w_\theta(\lambda, \mu, \theta) d\theta \right\} d\lambda d\mu \\
&= \alpha\gamma \int\int_{E^2} \sigma(\lambda, \mu) d\lambda d\mu \qquad (3.2) \\
&= \alpha\gamma \times \text{area of } f(E^2).
\end{aligned}$$

There are but a finite number of singular fibres in $M^3$. Let $q_1, \ldots, q_m \subset M^2$ be their images under $f$ and let $K^2$ be a triangulation of $M^2 - (q_1 \smile \ldots \smile q_m)$, which will be an infinite complex if there are multiple fibres. Let the simplexes in $K^2$ be so small that $T = f^{-1}(\tau^2)$ is a fibre neighborhood of $H = f^{-1}(q)$, for each 2-simplex $\tau^2$ in $K^2$ and any inner point $q \in \tau^2$. We may also suppose that each $\tau^2$ is in the domain of a local coördinate system for $M^3$. Then (3.1) is established by summing the equalities (3.2), with $T = f^{-1}(\tau^2)$, for each $\tau^2$ in $K^2$.

4. Now let a simple fibre, $H$, bound[4] a surface, $C$, which we assume to be piecewise differentiable. Let $C$ be given locally by $x^i = x^i(\xi, \eta)$, where $x^i(\xi, \eta)$ are differentiable functions of the parameters $\xi, \eta$. Then, by (1.2), (1.1) and Stokes' Theorem we have

$$\gamma = \int_H v_i dx^i = \alpha \int_C \int \sigma \left\{ \frac{\partial(\lambda, \mu)}{\partial(x^2, x^3)} \frac{\partial(x^2, x^3)}{\partial(\xi, \eta)} + \frac{\partial(\lambda, \mu)}{\partial(x^3, x^1)} \times \frac{\partial(x^3, x^1)}{\partial(\xi, \eta)} + \frac{\partial(\lambda, \mu)}{\partial(x^1, x^2)} \frac{\partial(x^1, x^2)}{\partial(\xi, \eta)} \right\} d\xi d\eta$$

$$= \alpha \int_C \int \sigma \frac{\partial(\lambda, \mu)}{\partial(\xi, \eta)} d\xi d\eta,$$

which is the degree of the map $f|C$. Hence $\gamma$ is the Hopf invariant of the map $f$, and (1.3) is established in case $f: S^3 \to S^2$ is a fibre mapping.

5. Let $S^2$ be given by $x^2 + y^2 + z^2 = 1$ and $S^3$ by $|\xi|^2 + |\eta|^2 = 1$, where $x, y, z$ are Cartesian coördinates for $R^3$ and $\xi, \eta$ are complex coördinates for $R^4$. Let $f: S^3 \to S^2$ be given by

$$x + iy = 2\rho\xi^m\bar{\eta}^n, \quad z = \rho(|\xi|^{2m} - |\eta|^{2n}),$$

where $\rho = 1/(|\xi|^{2m} + |\eta|^{2n})$ and $m, n$ are any (positive, zero or negative) integers, which are prime to each other (unless one or both is zero). Then, $f$ is a fibre map, with fibres given by[5] $\xi = \xi_0 e^{in\theta}$, $\eta = \eta_0 e^{im\theta}$, and its Hopf invariant[6] is $\pm mn$, the sign depending on the orientation of $S^3$. Thus $m$ and $n$ may be chosen, say with $m = \pm\gamma$, $n = 1$, so that $f$ has a given invariant, and is therefore homotopic[7] to a given map $S^3 \to S^2$.

6. We complete the proof of (1.3) by showing that the integral is an invariant of the homotopy class of the map $f$. This will be included in a more general result, which has no reference to fibre maps. Let $M^3$ and $M^2$ be twice differentiable manifolds and $f: M^3 \to M^2$ any twice differentiable map. Let $\omega_2$ be the form in $M^3$ which is given by (2.1), and assume that $\omega_2$ is an exterior derivative. Let $\omega_1' = \omega_2$, where $\omega'$ denotes the exterior derivative of $\omega$. Then we prove that

(*A*) *The integral*

$$I(f) = \int_{M^3} \omega_1 \square \omega_2$$

*is independent of the choice of the form* $\omega_1$, *satisfying* $\omega_1' = \omega_2$.

(*B*) *Let* $F: M^3 \to M^2$ *be any twice differentiable map, which is homotopic to* $f$. *Then the form* $\Omega_2$, *given by* (2.1) *in terms of* $F$, *is also an exterior derivative, and*

(*C*) $I(F) = I(f)$.

The statement (*A*) follows at once from the fact that, if $\omega_1' = \bar{\omega}_1' = \omega_2$, then $\bar{\omega}_1 - \omega_1$ is a closed form. Since $\omega_2$ is an exterior derivative, so is $(\bar{\omega}_1 - \omega_1) \square \omega_2$, whence

$$\int_{M^3} \bar{\omega}_1 \square \omega_2 - \int_{M^3} \omega_1 \square \omega_2 = \int_{M^3} (\bar{\omega}_1 - \omega_1) \square \omega_2 = 0.$$

In order to prove (*B*) and (*C*) we imbed $M^2$ as an analytic surface in $R^3$. Let $N$ be the open set consisting of points whose distance from $M^2$ is less than a positive $\rho_1$, which is so small that no two normals to $M^2$ intersect in $N$. Let $\rho > 0$ be so small that, if $\delta(q, q') \leq \rho$, where $q, q' \subset M^2$ and $\delta(q, q')$ is the Euclidean distance from $q$ to $q'$, then the linear segment, which is given in vector notation by $(1 - t)q + tq'$, for $-1 \leq t \leq 2$, lies in $N$. This being so, let $\phi(q, q', t)$ be the normal projection of $(1 - t)q + tq'$ on $M^2$.

It is clearly sufficient to prove (*B*) and (*C*) in case $\delta\{f(p), F(p)\} \leq \rho$ for each $p \in M^3$. Let this be so and let $M^4$ be the open manifold $M^3 \times (-1, 2)$. Then a twice-differentiable map, $g: M^4 \to M^2$, is defined by

$$g(p \times t) = \phi\{f(p), F(p), t\},$$

and $g(p \times 0) = f(p)$, $g(p \times 1) = F(p)$. Let $x^1, x^2, x^3$ be local coördinates for $M^3$ and let the map $g$ be given locally by $\lambda = \lambda(x^1, x^2, x^3, t)$, $\mu = \mu(x^1, x^2, x^3, t)$. Let $\bar{\omega}_2$ be the form in $M^4$, which is given by (2.1). Any cycle in $M^4$ is homologous to a cycle in $M^3 \times 0$, on which $\bar{\omega}_2$ reduces to the form, $\omega_2(0)$, associated with the map $f$. Since the latter is an exterior derivative its integral over any cycle in $M^3$ is zero. Therefore the integral of $\bar{\omega}_2$ over any cycle in $M^4$ is zero.

Let $M_0^4 = M^3 \times \langle 0, 1 \rangle$, so that $M_0^4$ is a bounded manifold in $M^4$. On p. 66, *et seq.*, of de Rham's paper let $A$ be a complex covering $M_0^4$. Then, with other minor adjustments in his arguments, it follows that there is a form $\bar{\omega}_1$, defined throughout an open set, $U \subset M^4$, which contains $M_0^4$, such that $\bar{\omega}_1' = \bar{\omega}_2$ in $U$. Since $M_0^4$ is compact we may take $U = M^3 \times (-\epsilon, 1 + \epsilon)$ for some $\epsilon > 0$.

Since $\bar{\omega}_1' = \bar{\omega}_2$ in $U$ we have $\omega_1'(t) = \omega_2(t)$ $(-\epsilon < t < 1 + \epsilon)$, where $\omega_1(t)$ and $\omega_2(t)$ are the forms which are obtained from $\bar{\omega}_1$ and $\bar{\omega}_2$ by writing $dt = 0$. This establishes (*B*), since $\omega_2(0) = \omega_2$, $\omega_2(1) = \Omega_2$, regarding $\omega_1(t)$ and $\omega_2(t)$ as forms in $M^3$, which depend on the parameter $t$.

In order to prove (*C*) it is sufficient to prove that $\partial\omega_3(t)/\partial t$ is an exterior derivative, where $\omega_3(t) = \omega_1(t) \square \omega_2(t)$ $(-\epsilon < t < 1 + \epsilon)$. For, treating $\omega_1(t)$, $\omega_2(t)$, $\omega_3(t)$ as forms in $M^3$, which depend on the parameter $t$, we have

$$\frac{d}{dt}\int_{M^3}\omega_1(t)\square\omega_2(t) = \int_{M^3}\partial\omega_3(t)/\partial t = 0$$

if $\partial\omega_3(t)/\partial t$ is an exterior derivative. To prove that it is we write $\bar{\omega}_3 = \bar{\omega}_1\square\bar{\omega}_2$. Then $\bar{\omega}_3' = \bar{\omega}_1'\square\bar{\omega}_2 = \bar{\omega}_2\square\bar{\omega}_2$, and it is clear from (2.1) that $\bar{\omega}_2\square\bar{\omega}_2 = 0$. Let $u_{abc}$ $(a, b, c = 1, \ldots, 4;\ x^4 = t)$ be the coefficients of $\bar{\omega}_3$. Since $\bar{\omega}_3' = 0$ we have

$$\frac{\partial u_{jk4}}{\partial x^i} - \frac{\partial u_{ik4}}{\partial x^j} + \frac{\partial u_{ij4}}{\partial x^k} - \frac{\partial u_{ijk}}{\partial t} = 0 \ (i, j, k = 1, 2, 3).$$

But $u_{ijk}$ $(i, j, k = 1, 2, 3)$ are the coefficients of $\omega(t)$. Therefore $\partial\omega_3(t)/\partial t = \theta_2'$, where $\theta_2 = u_{ij4}dx^i\delta x^j$, and the proof is complete.

7. We conclude with some additional observations. First notice that (3.2) is valid even if $H$ is a fibre of order $n > 1$. For, provided the total, area of $M^2$ is finite, (3.1) is true for open as well as for closed manifolds, the proof being the same in each case. Therefore (3.2), with $n > 1$, follows from (3.1), applied to the interior of $T$.

Also

$$\gamma = n\int_H \omega_1 \tag{7.1}$$

if $H$ is a fibre of order $n$. For if $H'$ is a simple fibre in a fibre neighborhood of $H$ it is obvious from the definition of a fibre neighborhood that there is a surface which is generated by fibres and bounded by $\pm(H' - nH)$. Therefore (7.1) is proved in the same way as (2.2).

Also if $H$ is a fibre of order $n$ and $E^2$ is a cross-section of a fibre neighborhood, $T$, of $H$, then $f$ maps $E^2$ on $f(E^2)$ with degree $n$. Combining these three results, we have

$$\begin{aligned}\int_T \omega_1\square\omega_2 &= \alpha\gamma \times \text{area of } f(E^2)\\ &= \alpha n\int_H \omega_1 \times \frac{1}{n}\int_{E^2}\sigma(\lambda, \mu)d\lambda d\mu\\ &= \int_H \omega_1 \times \int_{E^2}\omega_2\\ &= \int_H \omega_1 \times \int_m \omega_1,\end{aligned} \tag{7.2}$$

where $m$ is the boundary of $E^2$.

8. Let us now start with $\omega_1$, that is to say with a covariant vector-field $V$, which is defined all over $M^3$. Then curl $V$ is an alternating covariant tensor, which is the same as a contravariant vector density. Therefore "lines of flow" or "lines of force" are defined by curl $V$ and we assume them to be the fibres discussed in Section 2. Let $H$ be a given

fibre and $T$ a fibre neighborhood of $H$. Let $\lambda, \mu, \theta$ be the coördinates for $T$, which were defined in Section 3, except that we restrict $\theta$ to the interval $-1/n < \theta < 1/n$. Then these coördinates are valid even if $H$ is a multiple fibre, of order $n$. In these coördinates curl $V$ has components of the form $0, 0, u(\lambda, \mu, \theta)$, and

$$\frac{\partial u}{\partial \theta} = div(\text{curl } V) = 0.$$

From the transformation law of the components, $u_{ij}$, of curl $V$, namely

$$V_{ab} = u_{ij} \frac{\partial x^i}{\partial y^a} \frac{\partial x^j}{\partial y^b},$$

it follows that $\sigma(\lambda, \mu) = \mu(\lambda, \mu, \theta)$ is unaltered by coördinate transformations of the form $\theta' = \theta'(\lambda, \mu, \theta)$, $\lambda' = \lambda$, $\mu' = \mu$ and transforms in the same way as a scalar density in $M^2$ under transformations of the form $\lambda' = \lambda'(\lambda, \mu)$, $\mu' = \mu'(\lambda, \mu)$, $\theta' = \theta$. Hence curl $V$, whose components in the coördinates $(\lambda, \mu, \theta)$ are $0, 0, \sigma(\lambda, \mu)$, is related to the fibre mapping $(\lambda, \mu, \theta) \to (\lambda, \mu)$ by the equations (1.1), with the factor $1/4\pi$ discarded. In the notation of vector calculus (7.2) becomes

$$\int\int_T\int V \cdot \text{curl } V \, d\tau = \int_H V \cdot ds \times \int_M V \cdot ds.$$

[*] Hopf, H., *Math. Annalen*, **104**, 637–665 (1931).

[1] de Rham, G., *Journal de Math p. et a.*, **96**, 185 (1931).

[2] *Acta Mathematica*, **60**, 147–238 (1933).

[3] $\sigma > 0$ in positively oriented coördinate systems.

[4] In any case the fact that $\omega_2$ is an exterior derivative implies that $H$ bounds with division.

[5] Cf. Seifert, *loc. cit.*, p. 159.

[6] Let $H_1$, $H_2$ be fibres of orders $m$, $n$. Then $H \sim mH_1$ in $S^3 - H_2$ and $H' \sim nH_2$ in $S^3 - H$, where $H$, $H'$ are simple fibres near $H_1$, $H_2$, respectively, and it follows that $\gamma = \pm mn\, L(H_1, H_2)$, where $L$ denotes the linking coefficient. In this case $H_1$, given by $\eta = 0$, is of order $n$ and $H_2$, given by $\xi = 0$, is of order $m$, and $L(H_1, H_2) = \pm 1$ (cf. Hopf, *loc. cit.*, p. 655).

[7] Hurewicz, W., *Proc. Akad. Amsterdam*, **38**, 119 (1935). See also H. Freudenthal, *Compositio Math.*, **5**, 299–314 (1937).

# COHOMOLOGY INVARIANTS OF MAPPINGS

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The Hopf invariant[1] of a map of a $(2n-1)$-manifold on an $n$-manifold, and the Gysin[2] extension of the Hopf mechanism appear as special cases of the following general operation involving a map, cocycles and their products.

Let $K$, $K'$ be complexes, and $f\colon K \to K'$ a simplicial map. Then $f$ induces homomorphisms of the cochain groups and cohomology groups with integer coefficients. These are denoted by

$$f'\colon C^p(K') \to C^p(K), \quad f^*\colon H^p(K') \to H^p(K).$$

Suppose $u \in H^p(K')$, $v' \in H^q(K')$ are such that

$$f^*u = 0, \quad u \smile v = 0. \tag{1}$$

Choose representatives cocycles $u'$, $v'$ of $u$, $v$. By (1), there exist cochains $a \in C^{p-1}(K)$, $b \in C^{p+q-1}(K')$ such that

$$f'u' = \delta a, \quad u' \smile v' = \delta b.$$

It follows that

$$a \smile f'v' - f'b \tag{2}$$

is a $(p+q-1)$-cocycle of $K$. A different choice of $u'$, $v'$, $a$, $b$ usually alters the cohomology class of (2). However, it does so by an element of the subgroup of $H^{p+q-1}(K)$ generated by the two subgroups $f^*H^{p+q-1}(K')$ and $H^{p-1}(K) \smile f^*v$. Hence (2) defines a unique element

$$[f, u, v] \in H^{p+q-1}(K)/[f^*H^{p+q-1}(K') + H^{p-1}(K) \smile f^*v]. \tag{3}$$

In particular, if $f\colon S^3 \to S^2$ is a map of a 3-sphere on a 2-sphere, then any $u, v \in H^2(S^2)$ satisfy (1), and $[f, u, v] \in H^3(S^3)$. In case $u = v$ is a generator of $H^2(S^2)$, it can be proved that

$$[f, u, u] = \gamma z \tag{4}$$

where $z$ generates $H^3(S^3)$ and the integer $\gamma$ is the Hopf invariant of $f$.

This last result has a direct interpretation in tensor form by means of the de Rham theorem as formulated by Whitney.[3] Let $U$ be a covariant second order, alternating tensor field over $S^2$ whose integral over $S^2$ is 1. Assume $f$ to be differentiable, and let $f'U$ denote the field induced in $S^3$ by $U$ and $f$. Since the outer derivative of $U$ is 0, so also is that of $f'U$. Since the second Betti number of $S^3$ is 0, it follows from de Rham's theorem

that there exists a vector field $A$ over $S^3$ whose outer derivative is $f'U$. According to Whitney, the outer product $A \cdot f'U$ corresponds to the cup product $a \smile f'u$. Therefore, by (4), the integral $A \cdot f'U$ over $S^3$ is $\gamma$. This form of the result was obtained independently by J. H. C. Whitehead[4] who called my attention to the connection.

The principal property of the operation (3) is

1°. If $f$ is homotopic of $g$, then $[f, u, v] = [g, u, v]$.

This permits defining (3) for any continuous $f$ by the method of simplicial approximation.

2°. $[f, u, v]$ is linear in $u$. For $v$'s satisfying (1) and $f^*v = 0$, it is linear in $v$ and $[f, u, v] = (-1)^{pq}[f, v, u]$.

3°. If $f: K \to K'$, $g: K' \to K''$ and $u, v$ on $K''$ satisfy (1) with $g, K''$ in place of $f, K'$, then $u, v$ satisfy (1) with $gf, K''$ in place of $f, K'$ and

$$f^*[g, u, v] = [gf, u, v].$$

The left side is defined by choosing a representative of $[g, u, v]$ in $H^{p+q-1}(K')$, forming its $f^*$ image and reducing by the appropriate subgroup.

4°. If $f: K \to K'$, $g: K' \to K''$ and $u, v$ on $K''$ satisfy (1) with $gf, K''$ in place of $f, K'$, then $g^*u, g^*v$ satisfy (1) and

$$[f, g^*u, g^*v] = [gf, u, v] \mod [f^*H^{p+q-1}(K') + H^{p-1}(K) \smile f^*v].$$

5°. If $w \in H^r(K')$ and $u, v$ satisfy (1), then both $w \smile u, v$ and $u, v \smile w$ satisfy (1) and

$$f^*w \smile [f, u, v] = (-1)^r [f, w \smile u, v], \quad [f, u, v] \smile f^*w = [f, u, v \smile w].$$

The products on the right sides are defined by choosing representatives in the cohomology groups of $K$, multiplying, and then reducing by the appropriate subgroup.

The novel feature of the operation (3) is its use of those parts of the cohomology groups on which $f^*$ is trivial, namely: the kernel of $f^*$ and the factor group of $H^{p+q-1}(K)$ by the image of $f^*$. The operation is potentially richest in the case of just those maps heretofore called "algebraically inessential." It would seem appropriate to narrow the meaning of algebraically inessential to include only those maps $f$ for which $f^* = 0$ and each $[f, u, v] = 0$.

Several examples will indicate the ability of the operation to distinguish between maps of the same homology type.

*A*. Let $Y$ be composed of two circles $\alpha, \beta$ with a common point. Let $X$ be a circle, and let $f$ map $X$ into the commutator $\alpha\beta\alpha^{-1}\beta^{-1}$. Then $f$ is homologically trivial. However, if $u, v$ are generating 1-cocycles on $\alpha, \beta$, then $[f, u, v]$ generates $H^1(X)$.

*B*. Let $Y = S^2 \cup S^1$ be the union of a 2-sphere and a 1-sphere with a common point $y_0$. Let $X$ be a 2-sphere represented as a long tube $T$ with

two caps $C_1$, $C_2$. Orient $C_1$, $C_2$ concordantly and map each on $S^2$ with degrees $+1$, $-1$, respectively, and so that their boundaries are mapped on $y_0$. Map $T$ once around $S^1$. Then $f$ is homologically trivial. However, if $u$ is a generating 2-cocycle on $S^2$, and $v$ a generating 1-cocycle on $S^1$, then $[f, u, v]$ generates $H^3(X)$.

*C.* Let $Y$ be projective 3-space, and $f$: $S^3 \to Y$ the 2-fold covering. Then $H^2(Y)$ has one non-zero element $u$, and $[f, u, u]$ is the non-zero element of $H^3(S^3)/f^*H^3(Y)$.

*D.* Let $M$, $N$ be manifolds of dimensions $p$, $q$ and let $u$, $v$ be generating $p$, $q$-cocycles. Form $M \times N$. Let $m_0 \in M$, $n_0 \in N$, and let $Y = M \times n_0 \cup m_0 \times N$. Let $U$ be a closed normal neighborhood of $Y$ in $M \times N$, and let $F$ be the retraction of $U$ into $Y$. Let $X$ be the boundary of $U$ and $f = F|X$. Then $X$ is a $(p + q - 1)$-manifold and $[f, u, v]$ is its generating $(p + q - 1)$-cocycle. (Note that examples *A*, *B* are special cases of *D*.)

*E.* If, in *D*, $M$ and $N$ are spheres, then $X$ is a sphere, and $f$ represents the J. H. C. Whitehead product $[M, N]$.

The operation can also be defined for relative cohomology groups $H^p(K, L)$ (based on cocycles of $K$ which are 0 on $L$). Let $L_1$, $L_2$ be subcomplexes of $K$ and $L_3 = L_1 \cup L_2$; and similarly $K'$, $L_1'$, $L_2'$, $L_3'$. Suppose $f$: $(K, L_1, L_2) \to (K', L_1', L_2')$. Then $f$ induces homomorphisms

$$f_i^*\colon H^p(K', L_i') \to H^p(K, L_i) \quad i = 1, 2, 3.$$

Suppose $u \in H^p(K', L_1')$, $v \in H^q(K', L_2')$, $f_1^*u = 0$, $u \smile v = 0$ in $H^{p+q}(K', L_3')$. Then (2) defines a product

$$[f, u, v] \in H^{p+q-1}(K, L_3)/[f^*H^{p+q-1}(K', L_3') + H^{p-1}(K, L_1) \smile f^*v]. \quad (3')$$

A second extension involves the squaring operations $u \smile_i u$ introduced by me in a recent paper.[5] If $u \in H^p(K', L')$, then $u \smile_0 u = u \smile u \in H^{2p}(K', L')$ and $u \smile_i u$ $(i = 1, 2, \ldots)$ is an element of $H^{2p-i}(K', L')$ (reduced mod 2 if $p - i$ is even). Assuming $f^*u = 0$ in $H^p(K, L)$ and $u \smile_i u = 0$, choose as before $u'$, $a$, $b$ and form

$$a \smile_{i-1} a + a \smile_i f'u' - f'b. \quad (4)$$

Then (4) is a $(2p - i - 1)$-cocycle of $K$ which is 0 on $L$ (mod 2 if $p - i$ is even). Alteration of the choice of $u'$, $a$, $b$ varies the cohomology class of (4) by an element of the subgroup of $H^{2p-i-1}(K, L)$ spanned by $f^*H^{2p-i-1}(K', L')$ and squares of order $i - 1$ of elements of $H^{p-1}(K, L)$. Thus (4) defines

$$[f, u, v]_i \in H^{2p-i-1}(K, L)/[f^*H^{2p-i-1}(K', L') + Sq_{i-1}H^{p-1}(K, L)]. \quad (5)$$

(These groups are reduced mod 2 if $p - i$ is even.)

Now suppose $u \in H^p(L')$ and

$$\delta\colon H^p(L') \to H^{p+1}(K', L')$$

is the homomorphism induced by attaching to each cocycle of $L$ its coboundary in $K - L$. If $f^*u = 0$ in $H^p(L)$, and $u \smile_i u = 0$ in $H^{2p-i}(L')$, it follows that $f^*\delta u = 0$ in $H^{p+1}(K, L)$, and $\delta u \smile_{i+1} \delta u = 0$ in $H^{2p-i+1}(K', L')$. Thus

$$[f, u, u]_i \in H^{2p-i-1}(L)/[f^*H^{2p-i-1}(L') + Sq_{i-1}H^{p-1}(L)] \tag{6}$$

$$[f, \delta u, \delta u]_{i+1} \in H^{2p-i}(K, L)/[f^*H^{2p-i}(K', L') + Sq_iH^p(K, L)] \tag{7}$$

are defined. It follows now that

$$\delta[f, u, u]_i = [f, \delta u, \delta u]_{i+1} \tag{8}$$

where the left side is defined by choosing a representative, applying $\delta$, and reducing by the subgroup in (7).

If one starts with $f$: $S^3 \to S^2$, and the result of (4), then applies the operation of Einhängung and (8), the following result is obtained. If $f$: $S^{n+1} \to S^n$, and $u$ generates $H^n(S^n)$, then

$$[f, u, u]_{n-2} = \gamma z \in H^{n+1}(S^{n+1}) \mod 2 \tag{9}$$

where $z$ is the generator of $H^{n+1}(S^{n+1})$ and $\gamma = 0$ if $f$ is inessential, $\gamma = 1$ if $f$ is essential.

Let $L$ be a subcomplex of $K$, $f : L \to S^n$, and suppose the problem is to determine whether or not $f$ can be extended to a map $f'$: $K \to S^n$. Choose an $(n + 1)$-cell $E$ whose boundary is $S^n$. In any case $f$ extends to a map $F$: $(K, L) \to (E, S^n)$. Let $u$ generate $H^{n+1}(E, S^n)$. Then $F^*u \in H^{n+1}(K, L)$ and is precisely the primary obstruction[5] to the extension of $f$ to $f'$. If $F^*u = 0$, then $f$ can be extended to a map of the $(n + 1)$-skeleton of $K$ into $S^n$. Then a secondary obstruction $z^{n+2}(f)$ is obtained. We have shown that this is a unique element of

$$H^{n+2}(K, L)/Sq_{n-2}H^n(K, L) \mod 2.$$

Using (8) and (9), it can be proved that

$$z^{n+2}(f) = [F, u, u]_{n-1} \qquad n > 2.$$

Thus, if $\dim(K - L) = n + 2$, then $[F, u, u]_{n-1} = 0$ is a necessary and sufficient condition for the existence of the extension $f'$. If $\dim(K - L) > n + 2$, the vanishing of all $[F, u, u]_i$ $(i = 0, 1, \ldots, n)$ is a necessary condition for the existence of $f$. With more information concerning the homotopy groups of $S^n$, one might hope to prove it sufficient if $\dim(K - L) < 2n$.

Proofs of properties of $[f, u, v]$ based on the cochain definition (2) are long and cumbersome. The following second definition uses only well-established invariant properties of cohomology groups and products so that $[f, u, v]$ is invariant by definition. Proofs based on this definition are much simpler.

Let $X$, $Y$ be topological spaces, $f$ a map $X \to Y$, and let $u \in H^p(Y)$, $v \in H^q(Y)$ satisfy (1). Let $C$ be the mapping cylinder of $f$, and $F : C \to Y$ its projection. Let

$$i\colon X \to C, \qquad j\colon (C, 0) \to (C, X)$$

be identity maps. Since $F$ is homotopic to the identity map of $C$, $F^*$ is isomorphic. Let $\bar{u} = F^*u$, $\bar{v} = F^*v$. Then $\bar{u} \smile \bar{v} = F^*(u \smile v) = 0$. Furthermore $i^*\bar{u} = i^*F^*u = (Fi)^*u = f^*u = 0$. By exactness of the cohomology sequence of $(C, X)$, there exists a $u' \in H^p(C, X)$ such that $j^*u' = \bar{u}$. Form $u' \smile \bar{v} \in H^{p+q}(C, X)$. Then $j^*(u' \smile \bar{v}) = j^*u' \smile \bar{v} = \bar{u} \smile \bar{v} = 0$. By exactness of the cohomology sequence of $(C, X)$, there exists an element $w \in H^{p+q-1}(X)$ such that $\delta w = u' \smile \bar{v}$. An examination of the effect of choosing different elements $u'$, $w$ shows that $w = -[f, u, v]$ is unique mod$[f^*H^{p+q-1}(Y) + H^{p-1}X) \smile f^*v]$.

[1] *Math. Annalen*, **104**, 637–665 (1931); *Fund. Math.*, **25**, 427–440 (1935).

[2] *Comment. Math. Helv.*, **14**, 61–122 (1942).

[3] *Bull. Amer. Math. Soc.*, **43**, 785–805 (1937).

[4] See also "An Expression of Hopf's Invariant as an Integral," *Proc. Nat. Acad. Sci.*, **33**, 117–123 (1947).

[5] "Products of Cocycles and Extensions of Mappings," to appear in the *Annals of Math.*, No. 2 (1947).

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## THE $(L^2)$-SPACE OF RELATIVE MEASURE

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1. Let a real- or complex-valued function $f = f(x)$, where $0 < x < \infty$, be called of class $(N^2)$ if it satisfies the following conditions: $f(x)$ is of class $(L^2)$ on every bounded interval $(0, X)$ and the mean-value, $M(|f|^2)$, of $|f|^2$ exists as a finite limit, where the operator $M$ is defined by

$$M(g) = \lim_{X \to \infty} \int_0^X g(x)dx/X. \tag{1}$$

If $f$ and $g$ are of class $(N^2)$, then $\bar{M}(|f - g|^2) < \infty$, where

$$\bar{M}(p) = \limsup_{X \to \infty} \int_0^X p(x)dx/X, \qquad (p \geqq 0). \tag{2}$$

Since $\bar{M}(p_1 + p_2) \leqq \bar{M}(p_1) + \bar{M}(p_2)$, it follows that a metric function space, to be called the $(N^2)$-space, can be defined as follows: The elements of the space consist of all functions of class $(N^2)$, and two elements, $f$ and $g$, of the space are considered as identical if their distance is zero, with the under-

standing that the distance is defined as the non-negative square root of $\bar{M}(|f - g|^2)$.

It is clear that, when $f(x)$ is periodic (for $x > 0$), it is of class $(N^2)$ if and only if it is of class $(L^2)$ over a period, and that the metric of the space $(N^2)$ then becomes identical with the metric of the corresponding $(L^2)$-subspace. More generally, it is clear that, if almost-periodicity $(B^2)$ is referred to the half-line $x > 0$, then $f(x)$ is of class $(N^2)$ whenever it is of class $(B^2)$, and that the metric of the space $(N^2)$ becomes identical with the metric of its $(B^2)$-subspace. However, while both subspaces $(L^2)$, $(B^2)$ are linear vector spaces, it turns out that the full space $(N^2)$ is not linear. On the other hand, the completeness property of the subspaces $(L^2)$, $(B^2)$ (Fischer-Riesz, Besicovitch) proves to be shared by the full space $(N^2)$.

2. Let $f(x)$ be called of class $(N^2)_0$ if it is a bounded function of $x$ and is of class $(N^2)$, and let the metric space $(N^2)_0$ be defined as the function space consisting of the functions of class $(N^2)_0$ and carrying the metric of the $(N^2)$-space.

By considerations rediscovered by Besicovitch (1926), Nalli[1] (1914) proved that, if a sequence of elements in $(N^2)_0$ is a Cauchy sequence, then it has a limit in $(N^2)$. In addition, Nalli[2] emphasized that her result implies an analogue of the Fischer-Riesz theorem for the "orthogonal system" $e^{i\lambda x}$, $-\infty < \lambda < \infty$, of the $(B^2)$-space.

However, the $(N^2)_0$-space is not complete. In order to see this, it is sufficient to consider the sequence of partial sums of the Fourier series of any periodic function of class $(L^2)$ which fails to be bounded (even if $x$-sets of measure zero are disregarded).

3. *The space $(N^2)$ is not a linear space.*[3]

This is equivalent to the statement that $f_1$ and $f_2$ can be in $(N^2)$ when $f_1 + f_2$ is not. When $f_1$ and $f_2$ are real, this, in turn, is equivalent to the statement that the mean-value, (1), of the product $g = f_1 f_2$ of two functions of class $(N^2)$ need not exist. Hence, if $f_1$ is chosen to be the constant 1 and if $f_2$ is denoted by $f$, it follows that it is sufficient to ascertain the existence of a function $f$ of class $(N^2)$ for which $M(f)$ fails to exist. But such an $f$ results by choosing $f(x)$ to be $+1$ or $-1$ according as $x$ is or is not in $S$, where $S$ is a sequence of $x$-intervals having the following property: The mean-value $M(h)$ does not exist if $h$ is the characteristic function of $S$ (that is, $h(x)$ is 1 or 0 according as $x$ is or is not in $S$). Since the existence of such $S$ or $h(x)$ is obvious, it follows that $(N^2)$ is not linear.

*There does not exist in $(N^2)$ a linear subspace containing all linear subspaces of $(N^2)$.*

This contains the preceding fact (since otherwise the space $(N^2)$ itself would be an extremal linear subspace), but the converse deduction is also possible. In order to see this, it is sufficient to observe that the constant multiples of any $f$ contained in $(N^2)$ form a linear subspace of $(N^2)$.

4. *The space* $(N^2)$ *is complete.*

In other words, if $f_1, f_2, \ldots$ is any sequence of functions contained in $(N^2)$ and satisfying

$$\bar{M}(|f_j - f_k|^2) \to 0 \text{ as } j, k \to \infty, \tag{3}$$

then there exists in $(N^2)$ a function $f$ satisfying

$$\bar{M}(|f - f_j|^2) \to 0 \text{ as } j \to \infty. \tag{4}$$

The proof is a refinement, and at the same time a simplification, of the proofs of Besicovitch[4] and of Nalli.[5]

The limit relation (3) obviously implies the existence of an unbounded increasing sequence of positive numbers $R(1), R(2), \ldots$ such that

$$\lim_{n\to\infty} \sigma(n) = 0,$$

where

$$\sigma(n) = \text{fin sup}_{m>n,\, X>R(m)} \int_0^X |f_m(x) - f_n(x)|^2 dx/X, \tag{5}$$

if the fin sup refers to $m$, while $n$ is fixed. Let $n_1 < n_2 < \ldots$ be a sequence of integers satisfying

$$\sum_{k=1}^{\infty} \sigma(n_k) < \infty. \tag{6}$$

Let $g_k$ denote the $n_k$-th function of the sequence $f_1, f_2, \ldots$ and let $S(k) = R(n_k)$. Put $f(x) = 0$ if $0 < x < S(1)$ and $f(x) = g_k(x)$ if $S(k) \leqq x < S(k+1)$, where $k = 1, 2, \ldots$.

It will first be shown that

$$\bar{M}(|f - g_k|^2) \to 0 \text{ as } k \to \infty. \tag{7}$$

To this end, let $X$ be any number exceeding $S(k)$, where $k$ is fixed. If $i\ (\geqq k)$ denotes the integer for which $S(i) < X \leqq S(i+1)$, then

$$\int_{S(1)}^{X} |f(x) - g_k(x)|^2\, dx = \sum_{j=1}^{k-1} \int_{S(j)}^{S(j+1)} |g_j(x) - g_k(x)|^2 dx +$$

$$\sum_{j=k+1}^{i-1} \int_{S(j)}^{S(j+1)} |g_j(x) - g_k(x)|^2 dx + \int_{S(i)}^{X} |g_i(x) - g_k(x)|^2 dx.$$

Hence it is seen from (6) and the definitions of $g_j$ and $S(j)$, that

$$\int_{S(1)}^{X} |f(x) - g_k(x)|^2 dx/X \leqq \sum_{j=1}^{k-1} \int_{S(j-1)}^{S(j)} |g_j(x) - g_k(x)|^2 \cdot X + \sum_{j=k+1}^{i} \sigma(n_j).$$

Consequently, as $X \to \infty$,

$$\bar{M}(|f - g_k|^2) \leqq \sum_{j=k+1}^{\infty} \sigma(n_j),$$

and so (7) follows from (6).

It is clear that (4) follows from (7), and from the definition of $g_k$, in virtue of the triangular inequality.

It remains to be shown that $f(x)$ is of class $(N^2)$. Clearly, the existence of (1) for $g = |f_n|^2$, where $n = 1, 2, \ldots$, and (3) imply that

$$\mu = \lim_{n \to \infty} M(|f_n|^2) \tag{8}$$

exists as a finite limit. On the other hand, by Minkowski's inequality,

$$\left| \int_0^X |f(x)|^2 dx - \int_0^X |f_n(x)|^2 dx \right| / X \leqq \int_0^X |f(x) - f_n(x)|^2 dx / X; \tag{9}$$

hence, if the integer $n$ is fixed so that neither $|\mu - M(|f_n|^2)|$ nor $\bar{M}(|f - f_n|^2)$ exceeds a given $\epsilon > 0$, then, whenever $X$ exceeds a bound depending on $\epsilon$ and $n$,

$$\left| \int_0^X |f(x)|^2 \, dx/X - \mu \right| < 2\epsilon.$$

Consequently, (1) exists for $g = |f|^2$, and

$$M(|f|^2) = \mu. \tag{10}$$

5. Let a function $f(x)$, where $0 < x < \infty$, be called of class $(\bar{N}^2)$ if $f(x)$ is of class $(L^2)$ on every bounded interval $(0, X)$ and $\bar{M}(|f|^2)$ is finite. The functions of class $(\bar{N}^2)$ will be considered to form a metric space carrying the same metric as that of the $(N^2)$-space; so that the $(N^2)$-space is a subspace of the $(\bar{N}^2)$-space.

*The $(\bar{N}^2)$-space is linear and complete.*

The linearity of $(\bar{N}^2)$ follows from the inequality mentioned at the beginning (after the definition of $\bar{M}$). On the other hand, the completeness is a consequence of the above proof, even though not of the final wording, of section 4.

6. It is clear that the above considerations can be adapted to any of the spaces $(N^p)$, where $p \geqq 1$, which correspond to the space $(N^2)$ in the same way as the classes $(L^p)$ relate to the class $(L^2)$.

*If $p \geqq 1$ is arbitrary, the space $(N^p)$ is complete but not linear.*

It is understood that, if $p = 1$, the class $(N) = (N^1)$ is meant to be defined as consisting of those functions $f$ which are of class $(L) = (L^1)$ on every bounded $x$-interval and satisfy the condition that $M(|f|)$ exists as a finite limit.

If $p > 1$ and $p^{-1} + q^{-1} = 1$, then the product of two functions, one of which belongs to $(B^p)$ and the other to $(B^q)$, belongs to $(B) = (B^1)$. But

this becomes false if $(B^p)$, $(B^q)$ and $(B)$ are replaced by $(N^p)$, $(N^q)$ and $(N)$, respectively. This follows, even for $p = 2 = q$, from the above example proving that $(N^2)$ is not linear. Correspondingly, $(N^q)$ *cannot be interpreted as the dual space of* $(N^p)$, since such an interpretation would involve the definition of a scalar product.

7. Let $(\overline{N}^p)$ denote the space which relates to the space $(N^p)$ in the same way as the space $(\overline{N}^2)$ relates to $(N^2)$.

*If* $p \geqq 1$ *is arbitrary, the space* $(\overline{N}^p)$ *is complete and linear.*

The proofs are the same as before.

The situation can be summarized as follows: $(B^p)$ is a subspace of $(N^p)$ and $(N^p)$ is a subspace of $(\overline{N}^p)$; all three of these spaces are complete; the first and third of them are linear but the second is not.

[1] Actually, the definitions of Nalli (*loc. cit.*,[2] p. 306) are based on what results when the distance is assigned under the assumption that the upper limit occurring in (2) is replaced by the corresponding limit (which is required to exist). However, a glance at the proofs given by Nalli shows that this definition of her function space is equivalent to the above definition of the function space $(N^2)_0$.

[2] Nalli, P., *Rendiconti Circolo Matematico Palermo*, **38**, 307, 318–319, 322–323 (1914).

[3] This fact has curious methodical consequences for a problem relating to the Riemann zeta-function; cf. Wintner, A., *Duke Mathematical Journal*, **10**, 430 (1943), where the situation is explained in detail.

[4] Besicovitch, A. S., *Almost Periodic Functions*, Cambridge, 110–112 (1932).

[5] Nalli, P., *loc. cit.*[2] pp. 308–309.

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## *ON THE MAXIMUM PARTIAL SUM OF INDEPENDENT RANDOM VARIABLES*

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Let $X_n$, $n = 1, 2, \ldots$ be independent random variables with moments

$$E(X_n) = 0, \qquad E(X_n^2) = 1, \qquad E(|X_n|^3) = \gamma_n. \tag{1}$$

The reduction of the variance to 1 is not necessary, but is made here solely for the sake of simplicity. Let

$$S_n = \sum_{\nu=1}^{n} X_\nu, \qquad S_n^* = \operatorname*{Max.}_{1 \leq \nu \leq n} |S_\nu|$$

It is a trivial observation that the same law of the iterated logarithm holds for $S_n^*$ as for $S_n$. In particular one of Feller's theorems[1] becomes: If

$$\sup. |X_n| = 0\,(n^{1/2}(lg_2 n)^{-1/2}), \tag{2}$$

then ("*i. o.*" standing for "infinitely often").

$Pr(S_n^* > n^{1/2}[2lg_2n + 3lg_3n + 2lg_4n + \ldots + 2lg_{p-1}n + (2+\delta)lg_pn]^{1/2}$ *i. o.*) is zero or one according as $\delta$ is positive or not.

In the opposite direction Erdös has proved (oral communication) that there exist two constants $c_2 > c_1 > 0$ such that

$$Pr(c_1 < \liminf n^{-1/2}(lg_2n)^{1/2}S_n^* < c_2) = 1.$$

His method, of an elementary nature, seems incapable of a sharper result. We shall show that if, besides (1), we assume that $\gamma_n$ is e.g. bounded, then we can prove the following precise theorem which is the counterpart of Feller's theorem.

THEOREM. *We have*

$$Pr(S_n^* < \pi 8^{-1/2}n^{1/2}[lg_2n + 2lg_3n + lg_4n + \ldots + lg_{p-1}n + (1+\delta)lg_pn]^{-1/2} \text{ } i.\ o.)$$

*equal to zero or one according as $\delta$ is positive or not.*

The assumption that $\gamma_n$ is bounded can be considerably weakened; see the last paragraph of this abstract. However, the best possible condition has not been obtained.

We may also remark that the corresponding theorem for $S_n$ is radically different and has been treated by Erdös and the author.[2]

We shall sketch the main lines of our method in a series of lemmas.

Let $v_j = [jk^{-1}n]$, $j = 1, \ldots, k$. Then $(S_{v_1}, \ldots, S_{v_k})$ is a random point in a $k$-dimensional space. Let its distribution function be $F(u_1, \ldots, u_k) = Pr(S_{v_1} \leqq u_1, \ldots, S_{v_k} \leqq u_k)$. Lemma 1 is an extension of Liapounoff-Berry theorem[3] to $k$ dimensions and is proved by an extension of their methods. The condition regarding the third absolute moments is principally needed here. Notice that the dependence on $k$ is essential.

LEMMA 1. *We have*

$$|F(n^{1/2}u_1, \ldots, n^{1/2}u_k) - \Phi(u_1, \ldots, u_k)| \leq Q_1k^kn^{-1/2}$$

*where $Q_1$ (as $Q_2, \ldots$ later on) is a constant depending on the random variables but not on $k$, $n$, or the $u_j$'s; and $\Phi(u_1, \ldots, u_k)$ is the $k$-dimensional distribution function corresponding to the characteristic function*

$$\exp.\left(-\frac{1}{2k}\sum_{j=1}^{k}(t_j + \ldots + t_k)^2\right)$$

We shall write $\Phi(u) = \Phi(u, \ldots, u)$. Lemma 2 is an improvement on Erdös-Kac inequality.[4] The proof depends on Lemma 1 and also uses Liapounoff theorem.

LEMMA 2. *Let $c > 0$, $\psi_n > 0$ and $n/\psi_n \uparrow \infty$, $\epsilon_n \downarrow 0$. Then we have*

$$\Phi\left(\frac{c}{\sqrt{\psi_n}}\right)+\frac{Q_2k^k}{\sqrt{n}}>Pr\left(S_n^*<c\sqrt{\frac{n}{\psi_n}}\right)\geqq\Phi\left(\frac{c-\epsilon_n}{\sqrt{\psi_n}}\right)-$$
$$Q_2\left(\frac{k^k}{\sqrt{n}}+e^{-\frac{\epsilon_n^2k}{2\psi_n}}+\left(\frac{\psi_n}{\epsilon_n^2n}\right)^{1/3}\right)$$

In order to evaluate $\Phi(c\psi_n^{-1/2})$ we shall consider the special case where each $X_\nu = \pm 1$ with probability 1/2. In this case we find

LEMMA 3. *If* $\alpha_n = 0(\sqrt{n})$, *then*

$$Pr(S_n^*<\alpha_n\sqrt{n})=\frac{4}{\pi}\sum_{i=0}^{\infty}\frac{(-1)^i}{2i+1}\exp.\left(-\frac{(2i+1)^2\pi^2}{8\alpha_n^2}\right)+$$
$$0\left(\frac{1}{\alpha_n\sqrt{n}}\right)=T(\alpha_n)+0\left(\frac{1}{\alpha_n\sqrt{n}}\right),$$

*where* $T(\alpha_n)$ *is defined by the series.*

The proof of Lemma 3 starts with a combinatorial formula due to Bachelier,[5] and makes use of standard approximations together with a Fourier series transformation.

Combining Lemmas 2 and 3, and choosing, e.g.,

$$k=\frac{lgn}{8lg_2n}$$

we get

$$T\left(\frac{c+\epsilon_n}{\sqrt{\psi_n}}\right)+R_n\geqq Pr\left(S_n^*<c\sqrt{\frac{n}{\psi_n}}\right)\geqq T\left(\frac{c-\epsilon_n}{\sqrt{\psi_n}}\right)-R_n \quad (3)$$

where $T(\alpha)$ is defined in Lemma 3 and

$$|R_n|\leq Q_3\left(n^{-1/4}+\exp.\left(-\frac{\epsilon_n^2lgn}{16\psi_nlg_2n}\right)+\left(\frac{\psi_n}{\epsilon_n^2n}\right)^{1/3}\right). \quad (4)$$

We may remark in passing that from (3) and (4) we can deduce a remainder term to the limiting distribution of $S_n^*$, e.g., if $\alpha$ is a constant, then

$$Pr(S_n^*<\alpha\sqrt{n})=T(\alpha)+0\ (lg_2n(lgn)^{-1/2}).$$

Putting in (3) and (4) $\epsilon_n\psi_n^{-1/2}=\eta_n$ and choosing, e.g., $\eta_n \geqq 1600\ lg_2n\ (lgn)^{-1/2}$ we obtain

$$|R_n|\leq Q_4(lgn)^{-100}$$

If $\epsilon_n\psi_n = {}_0(1)$, it is easily seen that there exist two constants $A_2 > A_1 > 0$ such that

$$\frac{4}{\pi} e^{-\psi_n}(1 - A_1 \epsilon_n \psi_n) - \frac{4}{3\pi} e^{-9\psi_n} \leqq T\left(\frac{1}{\sqrt{\psi_n}}\left(\frac{\pi}{2\sqrt{2}} - \epsilon_n\right)\right) \leqq$$

$$T\left(\frac{1}{\sqrt{\psi_n}}\left(\frac{\pi}{2\sqrt{2}} + \epsilon_n\right)\right) \leq \frac{4}{\pi} e^{-\psi_n}(1 + A_2 \epsilon_n \psi_n).$$

These estimates can be sharpened to a certain extent, but they are sufficient for our present purposes. We state the main result as follows.

LEMMA 4. *If* $\psi_n \uparrow \infty$ *and*

$$\psi_n = 0(lg_2 n),$$

*then*

$$Pr\left(S_n^* < \frac{\pi}{2\sqrt{2}} \sqrt{\frac{n}{\psi_n}}\right) = \frac{4}{\pi} e^{-\psi_n}(1 + {}_0(1)).$$

Evidently the sequence,

$$\psi_n = lg_2 n + 2lg_3 n + lg_4 n + \ldots + lg_{p-1} n + (1 + \delta) lg_p n,$$

satisfies the conditions of Lemma 4.

Now we have the tools for proving our theorem. The proof for the case $\delta > 0$ follows easily from Lemma 4 by taking a sequence $n_k \sim \exp.(k/lgk)$. The proof for the case $\delta \leqq 0$ is much deeper and depends on the subtraction of a further subsequence with $k_v \sim 4vlgvlg_2 v$. We use in part arguments similar to Feller's, combined with estimates based on Lemma 4. It is impossible to indicate the method without going into details.

We state a more general theorem as follows. Let

$$s_n^2 = \sum_{v=1}^{n} \sigma_v^2, \qquad \Gamma_n = \sum_{v=1}^{n} \gamma_v$$

and make the following assumptions:

$$\Gamma_n = 0\left(s_n^2 \operatorname*{Max.}_{1 \leq v \leq n} \gamma_v^{1/3}\right)$$

$$\operatorname*{Max.}_{1 \leq v \leq n} \gamma_v^{1/3} = 0\left(s_n^{1-\vartheta}\right), \qquad \vartheta > 0$$

Let $\psi_n \uparrow \infty$, then

$$Pr\left(S_n^* < \frac{\pi}{2\sqrt{2}} \frac{s_n}{\sqrt{\psi_n}} \text{ i. o.}\right)$$

is equal to zero or one according as

$$\sum_{n=1}^{\infty} \frac{\sigma_n^2}{s_n^2} \psi_n e^{-\psi_n}$$

is convergent or divergent.

Detailed proofs of the above results will appear in another publication.

[1] Feller, W., "The General Form of the So-Called Law of the Iterated Logarithm," *Trans. Amer. Math. Soc.*, **54**, 373–402 (1943).

[2] Chung, K. L., and Erdös, P., "On the Lower Limit of Sums of Independent Random Variables," to appear in *Annals of Mathematics*.

[3] Berry, A. C., "The Accuracy of the Gaussian Approximation to the Sum of Independent Variates," *Trans. Amer. Math. Soc.*, **49**, 122–136 (1941).

[4] Erdös, P., and Kac, M., "On Certain Limit Theorems of the Theory of Probability," *Bull. Amer. Math. Soc.*, **52**, 292–302 (1946).

[5] Bachelier, *Calcul des probabilités*, t. 1, Paris (1912).

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## *SOME RESULTS ON ADDITIVE THEORY OF NUMBERS*

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Communicated March 31, 1947

Let $k$ be an integer $\geq 11$. Let $r_{2s}(P)$ be the number of solutions of the system of Diophantine equations

$$x_1^h + \ldots x_s^h = y_1^h + \ldots y_s^h, \quad 1 \leq h \leq k \tag{1}$$
$$1 \leq x\, y \leq P.$$

The author has proved that, for

$$s \geq k^2(2 \log k + \sqrt{\log k^2} + {}^1/_2 \log \log k + 4), \tag{2}$$

the asymptotic formula

$$r_{2s}(P) \sim c_1 c_2 P^{2s - {}^1/_2(k+1)}, \tag{3}$$

holds (for $P \to \infty$) where $c_1$ is a constant defined by the integral

$$c_1 = \int_{-\infty}^{\infty} \ldots \int_{-\infty}^{\infty} \left| \int_0^1 e^{2\pi i(\alpha_1 x^k + \ldots + \alpha_k x)} dx \right|^{2s} d\alpha_1 \ldots d\alpha_k$$

and $c_2$ by a singular series.

If $k < 11$, the formula (3) is still true for $s \geq s_0$, where $s_0$ is defined in the following table

| $k$ | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 |
|---|---|---|---|---|---|---|---|---|---|
| $s_0$ | 4 | 9 | 24 | 63 | 157 | 381 | 890 | 2035 | 4596 |

This is a precise information about Tarry's problem.

The author has previously[1] established an asymptotic formula for the number of solutions of the system of Diophantine equations with prime unknowns

$$p_1^h + \ldots + p_s^h = N_h, \qquad 1 \leq h \leq k.$$

He has now succeeded in extending its range of validity from

$$s \geq 4.14k(k+1)(k+2)\log k \qquad (s \geq 11)$$

to

$$s \geq k^2(4\log k + 2\sqrt{\log k^2} + \log\log k + 9) \qquad (s \geq 11). \quad (4)$$

Concerning Waring's problem Vinogradow[2] shows that the Hardy-Littlewood's asymptotic formula for the number of solutions of

$$x_1^k + \ldots + x_s^k = N, \qquad x_\nu \geq 0$$

holds for $s \geq 20\,k^2 \log k$. His method is hardly able to go beyond the order of magnitude $k^2 \log k$, but the numerical factor may still be improved. Indeed the author has replaced Vinogradow's inequality by a sharper one

$$s \geq 4k^2(\log k + {}^1/_2\sqrt{\log k^2} + {}^1/_4\log\log k + 1). \qquad (5)$$

Vinogradow's asymptotic formula for Waring-Goldbach's problem also holds within the range (5).

[1] Hua, *Additive Prime Number Theory*. This booklet was accepted for publication by the Acad. of USSR in 1940. The appearance was delayed by World War II.

[2] *Comptes Rendus of USSR*, 1942, No. 7.

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## *ON THE MULTIPLICITY OF STEADY GAS FLOWS HAVING THE SAME STREAMLINE PATTERN*

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The streamline pattern for any one steady flow of an ideal gas is the streamline pattern for a great many different modes of flow of such gas. Such trivial variants of the given flow as those obtainable by changing all pressures and densities in the same proportion are but special cases of wide classes of substitution flows having a common streamline pattern with the original flow. One such class of substitution flows of particular interest will be discussed here.

We shall consider steady flows of an ideal gas in which changes in entropy occur only in infinitely thin shock-front regions. This means that in regions between shocks the flow is *isentropic*, but not necessarily *homentropic*. That is, the entropy in a shock-free region is *constant along any given streamline* but is not necessarily *constant throughout the flow*. Only

if the flow is homentropic and not merely isentropic will the density be a unique function of the pressure throughout the shock-free region. The existence of such a function is the necessary and sufficient condition that the acceleration field, $-\frac{1}{\rho}\,\text{grad}\,p$, possess a potential (be irrotational). Since irrotationality of the acceleration field is necessary (but not sufficient) for irrotationality of the flow field, only such isentropic flows as are also homentropic may possibly be irrotational. Hence in considering flows which are isentropic but not necessarily homentropic we include rotational as well as irrotational flows.

We shall first limit our attention to regions between shock fronts, and consider that class of substitution flows for a given steady streamline pattern for which the pressures remain unchanged. That is for which

$$p' = p \tag{1}$$

Now the dynamic equilibrium of the force components normal to the streamline requires that the normal component of the pressure gradient balance the centrifugal reaction of the flow, or

$$\frac{\partial p'}{\partial n} = \frac{\rho' v'^2}{R} \tag{2a}$$

$$\frac{\partial p}{\partial n} = \frac{\rho v^2}{R} \tag{2b}$$

where $R$ represents the local radius of curvature of the streamline, $v$ the flow velocity and $\rho$ the mass density. Since $p' = p$, the condition

$$\rho' v'^2 = \rho v^2 \tag{3}$$

follows.

Now, for gases of constant specific heats, $C_p$ and $C_v$,

$$p \propto \rho^\gamma \exp.\,(s/C_v) \quad \begin{bmatrix} \gamma \equiv C_p/C_v \\ s = \text{entropy (specific)} \end{bmatrix} \tag{4}$$

For such gases the "velocity of sound" is given by

$$c \equiv \sqrt{\left(\frac{\partial p}{\partial \rho}\right)_s} = \sqrt{\frac{\gamma p}{\rho}} \tag{5}$$

and the Mach number, defined by

$$M \equiv \frac{v}{c} \tag{6}$$

becomes

$$M = \sqrt{\frac{\rho v^2}{\gamma p}} \tag{7}$$

Since $p' = p$ and $\rho' v'^2 = \rho v^2$, the additional condition

$$M' = M \tag{8}$$

is obtained.

A further condition restricting the possible substitution flows is the necessity of dynamic equilibrium of the force components *along* each streamline. That is to say, Bernoulli's equation must hold along each individual streamline:

$$\frac{\gamma}{\gamma - 1} p' + \frac{1}{2} \rho' v'^2 = H' \rho' \tag{9}$$

where $H'$ is a quantity (the "total enthalpy") which is constant along any particular streamline. The original flow satisfies a similar equation

$$\frac{\gamma}{\gamma - 1} p + \frac{1}{2} \rho v^2 = H \rho \tag{10}$$

Hence, from (1) and (3) it follows that

$$H' \rho' = H \rho, \text{ or } \rho' = \frac{H}{H'} \rho = m \rho \tag{11}$$

where $m$ is a parameter constant along any given streamline but variable from streamline to streamline. Since there are no further conditions to be imposed on the substitution flows, we still have at our disposal the value of the arbitrary parameter, $m$, for each streamline.

*The streamline pattern and all pressures and Mach numbers are left unchanged if along each streamline the values of density and of velocity are multiplied respectively by m and $1/\sqrt{m}$, where m may change from streamline to streamline.*

This class of substitution flows has thus far in our discussion been limited to shock-free regions, but this restriction can now be removed. Consider the flow quantities immediately in front of and immediately behind a shock front to be designated by the subscripts 1 and 2. Then the necessary conditions of conservation of mass, momentum and energy for a streamline passing through a steady shock front can be expressed in the form of equations (12–14):

$$\rho_1 v_1 = \rho_2 v_2 \tag{12}$$

$$\rho_1 v_1^2 \sin^2 \alpha_1 + p_1 = \rho_2 v_2^2 \sin^2 \alpha_2 + p_2 \tag{13}$$

$$\rho_1 v_1^2 + Kp_1 = \rho_2 v_2^2 + Kp_2 \qquad (14)$$

where $\alpha$ represents the angle between the streamline and the shock front, and $K$ is a constant depending on the kind of gas involved. It is clear that any substitution flow consistent with the requirements of equations (1), (3) and (11) will satisfy these shock conditions as well as does the original flow. Therefore this group of flow substitutions produces no change in streamline pattern even if shock fronts are present.

In the most general flows of an ideal gas the total enthalpy, $H$, ($\equiv i + \frac{v^2}{2}$, where $i$ is the specific enthalpy) is constant along each particular streamline, whether or not it intersects shock fronts, but it varies from streamline to streamline. The entropy is constant along each particular streamline in regions between shock fronts, but varies from streamline to streamline and increases discontinuously whenever the streamline crosses a shock front.

In supersonic regimes ($v > c$) flows of this most general character can be computed by the use of the general method of characteristics combined with the shock-front integral relations (equations (12–14)) when suitable boundary conditions are imposed. However, the computation is considerably simpler if either the total enthalpy, $H$, or the entropy, $s$, is universally constant for a region under consideration. For if $H$ is constant throughout the flow, a universal relation exists between $v$ and $c$, as from (5) and (10):

$$\frac{c^2}{\gamma - 1} + \frac{v^2}{2} = H \qquad (15)$$

On the other hand, if $s$ is constant throughout a region of the flow, there exists a universal relation between $p$ and $\rho$. (See equation (4).)

By the use of the class of substitution flows discussed above, a flow of non-constant $H$ and $s$ can be readily replaced by a substitution flow having either constant $H$ or (in a shock-free region) constant $s$ if the values of $m$ are suitably chosen on any surface intersecting each streamline a single time. The simpler substitute flow would then yield directly the pressures, Mach number and flow pattern of the original flow and, after a simple inversion of the conversion from original to substitute flow, the densities and velocities as well.

Similarly, if it is desirable to do so, an original flow problem involving non-constant $H$ and constant $s$ could be replaced by one having constant $H$ and non-constant $s$, and vice versa. It is not possible, however, to substitute a flow having *both* $H$ and $s$ constant for one in which either is non-constant. In other words, this class of substitution flows does not in general allow the replacing of a rotational flow by an irrotational one.

The relations discussed may be arrived at more briefly, but less directly, from dimensional considerations. Along each stream tube; there exists for a perfect gas only one independent dimensional reference quantity, as for instance the "reservoir" pressure. All variables can be expressed in terms of that reference pressure in conjunction with one non-dimensional quantity as for instance the local Mach number. Thus the local pressure is equal to the reference pressure multiplied by a function of the Mach number, and of non-dimensional quantities representing the geometry involved. Hence the lateral pressure gradient is determined and invariant with respect to transformations not involving changes of the geometry or changes of the reference pressure and thus changes of any pressure. It follows that it must be possible to write all pertinent equations in terms of the local pressure; the local Mach number and the space coördinates, thus eliminating one dependent variable.

Among possible flows calling for application of these relations, there are jets from different "reservoirs" flowing together. Such is the flow when a propulsion jet issues into the rapidly moving air (relative to a missile or airplane) of the atmosphere. It may also be instructive to idealize boundary layer wakes by considering them as jets of a perfect gas with a lowered total enthalpy.

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## *TURBULENCE AS AN ENVIRONMENTAL DETERMINANT OF RELATIVE GROWTH IN DAPHNIA*

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Temporal variation or cyclomorphosis is pronounced in many limnetic races of *Daphnia*. The relative length of the head is the most variable aspect of such races of the north temperate zone. The winter and early spring generations bear short round heads resembling those characteristic of pond *Daphnia*. Individuals of midsummer generations have elongate heads, called helmets, which are often nearly as long as the rest of the body. During the period of these striking phenotypic changes reproduction is entirely a sexual, which means that the genetic constitutions of all generations are almost identical. These phenotypic differences must therefore be determined by seasonally variable cytoplasmic or environmental factors, in all probability the latter.

The aim of the present investigation is precise determination of all of the environmental factors controlling cyclomorphosis. The efforts of

students in the first two decades of this century were directed toward finding a single environmental variable which controlled the relative head size. Wesenberg-Lund[1] concluded that temperature was the controlling factor while Woltereck[2], denying this, claimed that the level of nutrition was the prime control. The experiments of Coker and Addlestone[3] in 1938 indicated, however, that different environmental factors were effective at different periods of the life history of an individual. They showed that temperature did control head size, but only during the embryonic period, some unisolated factor being involved during postnatal life. The author[4] was able to confirm these findings of Coker and Addlestone on several species.

The rate of relative helmet growth during postnatal life can be measured by the value of $k$ in Huxley's[5] allometric equation, $y = bx^k$, as was demonstrated in the study[4] of the population of *D. retrocurva* Forbes in Bantam Lake, Connecticut (1945). From April until mid-June head ($y$) and carapace ($x$) grew at the same relative rate ($k = 1$). Thereafter, helmet growth was tachyauxetic, the rate reaching a maximum ($k = 1.44$) a month later. It was still greater than unity ($k = 1.27$) at the end of the series in mid-August. Tachyauxetic growth occurred that season only when the water was above 18–19°C., but above this threshold appeared to be independent of both temperature and nutrition. Individuals of this population were cultured in laboratory vessels under conditions of temperature and nutrition which supported tachyauxetic helmet growth in the lake. Yet the helmets always grew relatively more slowly ($k = 0.52$ to 0.74) than the rest of the body in these laboratory cultures. An investigation of the effects of size of vessel[6] and population density[4] proved that they are not responsible for this difference between the rates of relative helmet growth in the lake and in laboratory cultures. An experimental study of the rôle of turbulence was more fruitful and forms the subject of this report.

The population of *D. retrocurva* in Bantam Lake was so small in the summer of 1946 that it was impossible to collect any experimental animals of this species. The substitution of another species was necessary, so a race of *Daphnia longispina* with apicate helmets, also present in Bantam Lake, was utilized. The shape of the helmets of this race in mid-summer can be judged from the right-hand column of figure 2. Individuals from this population were used in the investigation of the influence of population density and size of vessel, as well as that of turbulence on the rate of postnatal relative helmet growth. Some of the *Daphnia* collected on September 2 were preserved and the remainder were brought back alive to the laboratory. Twenty adults with eggs or embryos in the broodpouch were selected as parents. These adults were divided into two equal groups. One was to be maintained in turbulent water while the control group was kept in

the usual quiet water of laboratory culture vessels. The cultures were kept in two similar rectangular museum jars of dimensions 20 × 23 × 30 cm. Each held ten liters of Bantam Lake water, which had been twice filtered through 10 × bolting silk. The jars were placed side-by-side under fluorescent lights in a room with a fairly constant temperature. A small electric stirrer rotated a nearly straight glass rod which extended about ten centimeters below the surface of the water. The turbulence so produced was judged to be sufficient when the *Daphnia* were swept around by the current. Measurement of the amount of turbulence was not possible. The nannoplankton of the lake water reproduced rapidly enough to serve unaugmented as a sufficient source of food.

Periodic microscopic examination of the adults showed that all of them lived and reproduced in each culture. No measurements of their offspring were made during the first two weeks. The temperature fluctuated slowly between 17.2°C. and 23.1°C. The mean of the daily readings for the turbulent culture was 19.4°C., for the control 19.3°C. At the end of this period those offspring of each culture which had matured were measured and drawn.

The difference in relative head length in the two cultures was remarkable. While the heads in the controls were of the same relative length as in all previous laboratory cultures, those reared in turbulent water had relatively longer helmets, more nearly like those found in the lake. A representative adult from this first generation of each culture is drawn in figure 2.

Accumulation of sufficient data for a determination of the relative rates of helmet growth in the two cultures was highly desirable. All the instars of the first generation constituting the population at the end of the period could not be grouped to provide this information as the temperature, hence the relative head length at birth, had been too variable. It was therefore necessary to attempt a more rigorous temperature control. The two vessels were placed side-by-side in a 60-liter water bath. First generation adults were used in this experiment. Eight from the turbulent culture were replaced in turbulent water. Ten from the control culture were replaced in non-turbulent water. The temperature in each remained between 19.4°C. and 22.4°C. during this sixteen-day experimental period, except on the first day when it reached 23.0°C. Temperature fluctuations were simultaneous in the two cultures. The mean of the sixteen daily temperature records in each culture was 21.2°C. Nutrition as judged by the color of the guts was very good during the early phase of the experiment and slowly decreased so that at its termination, although adequate, it was poorer than at the outset. This change followed the same course in both vessels. It should be emphasized here that the relative rate of helmet growth is not influenced by changes in the nutritive level.

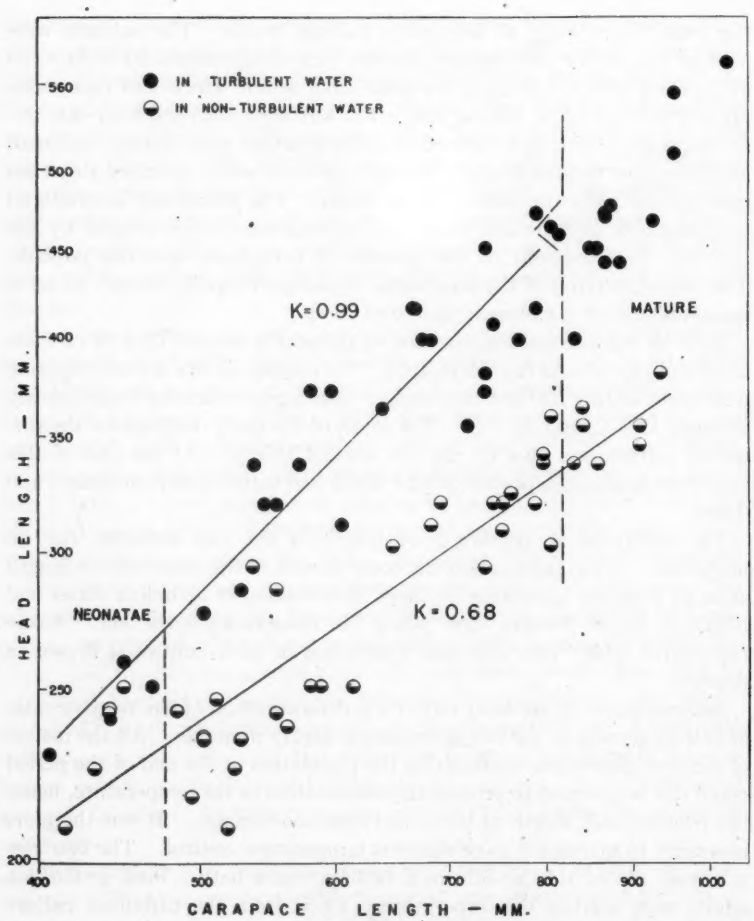


FIGURE 1.

Comparison of rates of relative head growth (*Daphnia longispina*, Bantam Lake, Conn.) in laboratory cultures with turbulent and non-turbulent water. See text for details.

Periodically a portion of the *Daphnia* population was removed from the culture vessels for examination. Each specimen was anesthetized with ethyl urethane, measured, and drawn with the camera lucida. These animals if properly treated, appeared to suffer no ill effects and were returned to the cultures. The measurements of head length and carapace length were made with an ocular micrometer. The head length was then plotted on a logarithmic grid against carapace length for each individual.

This gives a linear array of points for each culture. Measurement of the slope of the line of best fit for each array provides the $k$ value for each culture. The details of measuring and analysis of measurements are given elsewhere.[4] When the pre-adult rate of relative helmet growth is isauxetic or tachyauxetic, there is an appreciable fall in the rate after maturity. No change in rate is apparent when early growth is bradyauxetic. The exponent, $k$, for immature specimens in the turbulent water is 0.99. Calculated for all specimens in non-turbulent water it is 0.68. (Fig. 1.)

This difference between the rates of relative helmet growth in turbulent and non-turbulent cultures could be either a direct effect of turbulent water movements on the *Daphnia* or an indirect one. Turbulence might act indirectly by increasing nutrition or oxygen concentration. Yet, as pointed out above, the difference between the levels of nutrition in the two cultures at any time was small when judged by microscopic examination of the guts of the transparent living animals. Oxygen determinations using the Winkler process showed that the water in both vessels was saturated with oxygen at the end of the experiment. This leaves a direct effect of turbulence as the more likely explanation, but no definite information is available as to the manner in which it influences the growth processes of *Daphnia*. It is possible that there are environmentally conditioned differences in metabolic rate associated with different rates of relative helmet growth. This would parallel the situation in *Locusta migratoria* where the externally determined solitary and migratory phases are known to have significantly different rates of oxygen consumption.[7]

Adult *Daphnia* reared in turbulent water in the laboratory have helmets relatively larger than those reared in non-turbulent cultures but they do not attain the proportions of adults which developed in the lake at about the same temperature. A comparison of development in these three environments is given in figure 2. These camera lucida drawings are all to the same scale, except for the neonatae which have twice the linear magnification. The left and center columns contain specimens taken from the non-turbulent and turbulent laboratory cultures, respectively, while that on the right shows specimens in the Bantam Lake population at the time when the parents for the experimental cultures were collected. These parents had the appearance of the adult at the top of that column. The adults of similar size which head the other two columns are the offspring of these females which were reared under turbulent and non-turbulent laboratory culture. Development in the two laboratory cultures will first be considered. The relation of each specimen to the rest of the population samples can be checked in figure 1. More than one specimen is drawn for most size groups to illustrate the range of variation. Specimens toward the left of either column have relatively smaller helmets, appearing below the line of best fit in figure 1. Those to the right have relatively larger helmets

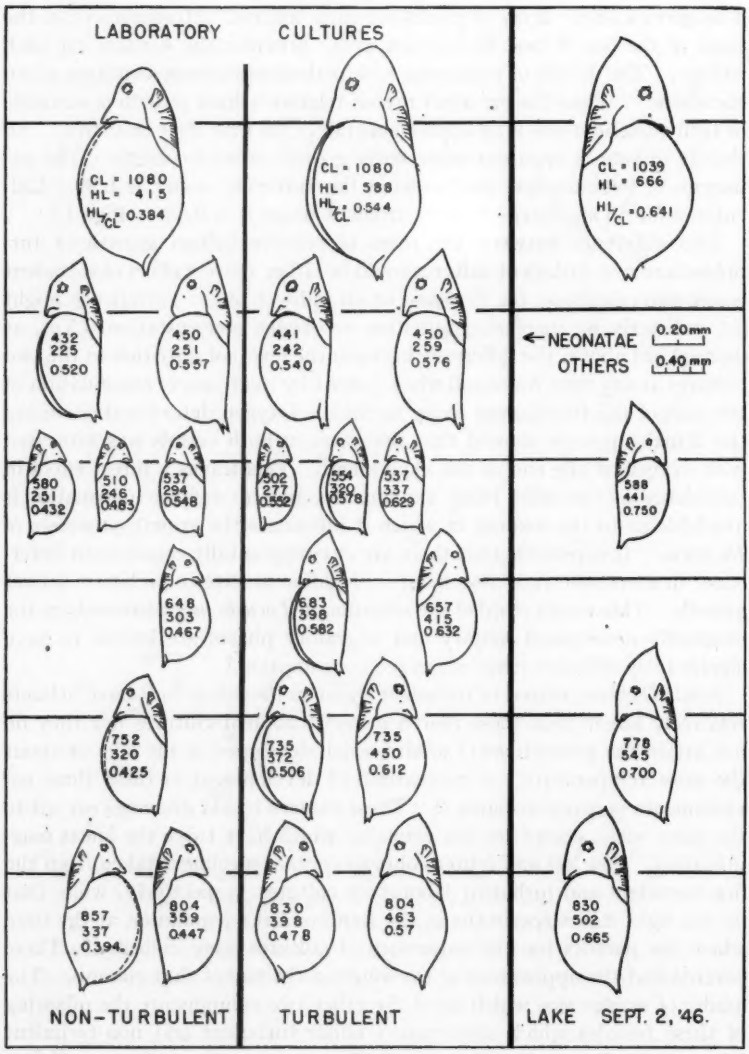


FIGURE 2.

Camera lucida drawings illustrating differences in helmet development in individuals from the same population of *Daphnia longispina* (Bantam Lake, Conn.) when reared in three different environments. The range of temperature and nutritive conditions was the same in all three. See text for details.

and those in the middle correspond to points on the curve. The relative helmet size of each *Daphnia* is given as a ratio. The effect of the different relative growth rates is apparent even on comparison of the neonatae with the next larger size group. While the newborn of the two cultures are identical, the lowest HL/CL ratio for specimens of the next larger size group in the turbulent culture is as high as the highest among the same sized individuals of the control culture. Among larger specimens the lowest HL/CL ratios in the turbulent culture are greater than the highest found in the same size group from non-turbulent water. The younger individuals in the right-hand column represent the only specimens of the appropriate instars to be found in the sparse population in Bantam Lake at that time. Unfortunately no neonatae were taken. As the temperatures at the surface, at one meter's depth and just above the bottom were 22.7°C., 21.6°C. and 20.2°C., respectively, the neonatae undoubtedly had helmets of about the same relative size as those in the laboratory cultures. If so, the helmets must have been growing relatively more rapidly than those in the turbulent laboratory culture in order to produce the higher helmets in the later instars. Although the absence of representatives of the early instars precludes an accurate determination of the relative growth rate, comparison with the laboratory growth rates should provide an approximation. The helmets in the turbulent laboratory culture were growing at the same relative rate as the rest of the body (isauxesis) since the slope of the line best fit is essentially unity ($k = 0.99$). Relative helmet grow thin the lake must therefore, have been tachyauxetic ($k > 1$).

The information which this experiment provides concerning the relative rates of helmet growth under different environemntal conditions is incomplete, yet it invites integration with what we know of the seasonal differences in relative growth rates in *D. retrocurva*. The helmets of the Bantam Lake population of the latter species grew isauxetically from the first of April to the middle of June 1945, under a wide range of nutritive and thermal conditions. The race of *Daphnia longispina* in the turbulent laboratory culture, well fed and at somewhat higher temperatures exhibited isauxetic helmet growth. This suggests that turbulence of the lake waters may be the factor permitting isauxesis. If so, it seems probable that turbulence is also necessary for tachyauxetic growth. Differences in the amount of turbulence might determine the rate of relative helmet growth or turbulence may be a necessary, but not a sufficient environmental factor for tachyauxesis. Further laboratory experimentation will explore this possiblity. There is also hope of estimating the seasonal variation in the amount of turbulence in lakes.

One remark about the significance of turbulence in the interpretation of the biology of limnetic *Daphnia* should be made. Should turbulence prove to be a necessary environmental condition for the development of helmets

in many races it would explain the oft-noted restriction of helmeted populations to the upper, turbulent waters of stratified lakes. Those living in lower, less turbulent waters usually have shorter helmets.

Summary.—Preliminary experiments on a cyclomorphic race of *Daphnia longispina* indicate that turbulence of the water is one of the environmental factors controlling relative rate of helmet growth during postnatal life.

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## *A DIRECT DEMONSTRATION OF THE PHOSPHORUS CYCLE IN A SMALL LAKE**

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It is well known that the total quantity of plankton present in the waters of a lake may undergo marked and rapid variation, so that in the course of a year a number of pulses or maximum populations may succeed each other. Juday and Birge[1] noted that such rises in the phytoplankton might occur without reducing the phosphate content of the water and that on occasions both phosphate and plankton might rise together. It has also been noted for example by Pearsall,[2] that rises in the population of blue-green algae may occur at the end of summer when it would seem that the phosphorus content of the water was totally inadequate to support an increased phytoplanktonic population. In an earlier paper[3] much indirect evidence was assembled indicating that in Linsley Pond, a small inland lake which develops a very stable thermal statification in summer, there is a continual liberation of phosphorus from the mud into the free water. Such of this phosphorus as enters the illuminated layers of the lake is believed to be taken up by the phytoplankton, later to be sedimented as a fine rain of particulate matter, partly no doubt dead phytoplankton, but also feces of

zooplankton feeding on the plant cells. The resultant movement of phosphorus is thus believed to be a horizontal movement into the free water as phosphate, and a vertical downward movement as seston or particulate matter. The very low concentrations usually observed in summer in the surface waters of lakes are thus to be regarded as steady state concentrations, maintained at low levels by the activity of the phytoplankton, the rate of development of which depends rather on the rate of supply of phosphate ions from the mud than on their concentration in the free water. Such an hypotheses explains the rather paradoxical situations encountered by other workers and is in accord with the facts relating to chemical cycles in Linsley Pond and other lakes. If the hypothesis is correct, phosphorus present in the surface as phosphate on any given day should move to greater depths during the course of the succeeding few days or weeks, even in fully stratified lakes in which virtually no mixing is taking place. The possibility of obtaining relatively large amounts of the radioactive isotope of phosphorus $P^{32}$ permits the hypothesis to be tested.

On June 21, 1946, a sample of radiophosphorus, of strength approximately ten millicuries, received as phosphoric acid and made up as sodium phosphate in hundredth normal sodium bicarbonate, was introduced into the surface water of Linsley Pond in twenty-four approximately equal portions. Twelve of the portions were placed at approximately equal distances along a line running across the middle of the lake from West to East. The other twelve samples were placed along a line between the first line and the outlet at the south end of the lake. As a light south wind was blowing at the time, and as the surface water of the lake could be seen to be drifting northward when the boat was anchored in the middle of the lake, it was believed that this distribution of the samples would secure a fairly uniform dispersion of radiophosphorus in the circulating surface waters.

Collections of water were made on June 28 in the deep central part of the lake normally used for limnological stations. For measurement of radioactivity, the vertical water column was divided into four layers, from each of which approximately 18 liters of water was collected, as is indicated in table 1. The depths of collection refer to the position of the top of the 1.25-liter Nansen reversing bottle used to collect the water, the figures after the depths indicate the number of times the bottle was filled at each depth. The deepest water to be included at each filling of the bottle came from approximately 50 cm. below the top of the bottle so that the composite samples may be regarded as representing I, 0–3 m.; II, 3–6 m.; III, 6–9 m. and IV, 9–14.5 m. layers. The composite sample from each layer was evaporated almost to dryness, the organic matter oxidized with nitric and perchloric acids, and after addition of a drop of phosphoric acid as a carrier, the phosphate was precipitated as ammonium phosphomolyb-

date. The measurements of radioactivity were performed on the dry phosphomolybdate precipitates on filter paper.

Owing to the great dilution of the radioactive material, it was necessary to make a large number of counts, particularly on the sample from layer III, and to test the significance of the means obtained by Fisher's[4] table of $t$. The voltage stabilizer of the only Geiger counter circuit available was not sufficiently good to prevent alterations in the background count which completely obscured the increases, in single two-minute counts, due to the radioactivity of the samples. In the case of the samples from layers I and II, the mean count was found to be significantly different from the background when twenty-one 2-minute counts had been made; for quantitative determination nine more counts were included. For the lower activity of the sample from layer III, sixty-three runs of two minutes, alternated with background counts on a control sample of phosphomolybdate

TABLE 1

| | LAYER | COMPOSITION OF SAMPLE | COUNTS PER M.$^3$ PER MIN. | PROBABILITY DUE TO CHANCE | VOL. LAYER M.$^3$ | COUNTS PER LAYER PER MIN. |
|---|---|---|---|---|---|---|
| I | 0–3 m. | 0.0 m., 2; 0.5 m., 3<br>1.0 m., 3; 1.5 m., 2<br>2.0 m., 3; 2.5 m., 2 | 1100 | 0.01–0.001 | 241,800 | $266.10^6$ |
| II | 3.6 m. | 3.0 m., 2; 3.5 m., 2<br>4.0 m., 3; 4.5 m., 2<br>5.0 m., 3; 5.5 m., 2 | 980 | 0.01–0.001 | 192,400 | $189.10^6$ |
| III | 6–9 m. | 6.0 m., 2; 6.5 m., 2<br>7.0 m., 3; 7.5 m., 2<br>8.0 m., 3; 8.5 m., 2 | 620 | 0.02–0.01 | 120,400 | $75.10^6$ |
| IV | 9–14.8 m. | 9.0 m., 4; 10.0 m., 3<br>11.0 m., 2; 12.0 m., 2<br>13.0 m., 2; 14.0 m., 1 | (200 | 0.5–0.6) | Sum of significant values | $530.10^6$ |

precipitated in concentrated oxidized surface water collected on June 13, 1946, were made. The counts for layer III only appear significant if the significance of the mean difference between each run and the immediately preceding control background run be considered. This is reasonable, since the voltage will vary little between two consecutive counting periods, but over the whole sixty-three pairs of observations the over-all changes in voltage will greatly increase the variance of both background and sample counts. The sample from layer IV gave no significant indication of radioactive phosphorus after forty-two observations had been made and must be regarded as negative. With improved counting facilities and a somewhat larger quantity of radiophosphorus it may be possible to measure the activity of phosphate phosphorus, organic soluble phosphorus and sestonic phosphorus spearately and to follow the cycle for longer periods on some future occasion.

The results of the study are set out in table 1, all counts being adjusted for decay and fluctuation of the counting rate, to correspond to the time and conditions of measurement of a phosphomolybdate preparation of an aliquot of $10^{-6}$ of the original sample, which gave a count above the background of 715 per minute on July 8. On this basis the total quantity of $P^{32}$ put into the lake corresponds to $715.10^6$ counts per minute. The total recovery therefore amounts to 74.2% of the $P^{32}$ introduced.

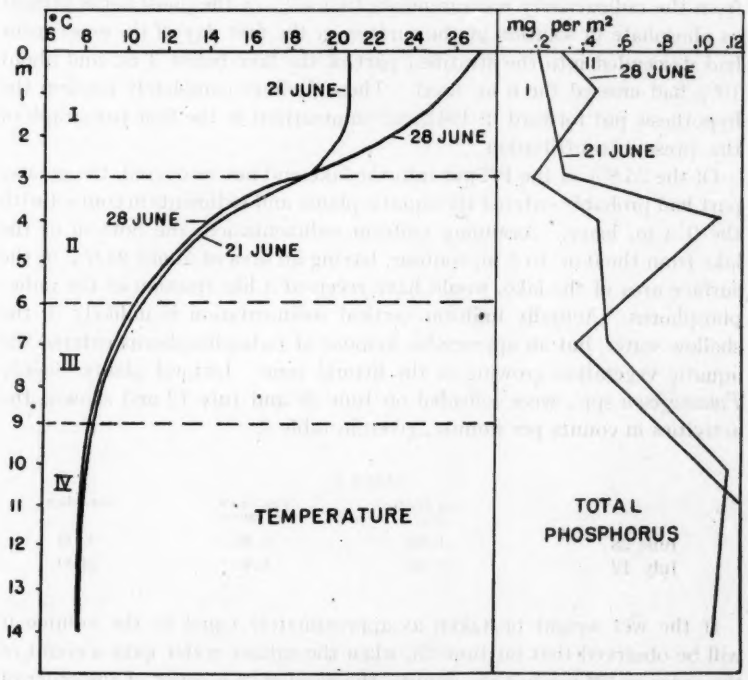


FIGURE 1.

Vertical distribution of temperature and total phosphorus in Linsley Pond 21 and 28 June, 1946, and limits of the four layers used in the present study.

The temperature curves (fig. 1) for the days at the beginning and end of the experiment are almost identical below three meters. The slightly lower temperature in the deep water on June 28 is certainly insignificant, and due to slight distortion of the isotherms by wind, as frequently occurs in all lakes. The chief change in the week of the experiment was a great heating of the surface water due to a sudden spell of hot weather.

Determinations of total phosphorus indicate a movement of phosphorus into the epilimnetic layer I during the period under consideration. The apparent decline in the total phosphorus at 4 m. in layer II is probably due to the descent of decomposing plankton which was clearly visible in the 4 m. sample on June 21, but which was probably at a greater depth and was consequently missed in sampling on June 28.

In spite of the fact that phosphorus has entered the epilimnion during a period when there can have been practically no vertical mixing, it is clear from the radioactivity measurements that 47% of the phosphorus present as phosphate in solution at the surface on the first day of the experiment had descended into the stratified part of the lake below 3 m. and about 10% had crossed the 6 m. level. These findings completely confirm the hypothesis put forward in 1941 and summarized in the first paragraph of the present contribution.

Of the 25.8% of the $P^{32}$ put into the lake and not recovered, the greater part had probably entered the aquatic plants and sediments in contact with the 0–3 m. layer. Assuming uniform sedimentation, the bottom of the lake from the 0 m. to 3 m. contour, having an area of about 24.6% of the surface area of the lake, would have received a like fraction of the radiophosphorus. Actually uniform vertical sedimentation is unlikely in the shallow water, but an appreciable amount of radiophosphorus entered the aquatic vegetation growing in the littoral zone. Littoral plants, mainly *Potamogeton* spp., were collected on June 28 and July 12 and showed the activities in counts per minute, given in table 2.

TABLE 2

| | PER GRAM WET | PER GRAM DRY (80°C.) | PER GRAM P. |
|---|---|---|---|
| June 28 | 1.65 | 8.85 | 4700 |
| July 12 | 0.68 | 4.47 | 2800 |

If the wet weight be taken as approximately equal to the volume it will be observed that on June 28, when the surface water gave a count of the order of $10^3$ per m.$^3$ per minute, the weed gave a count of the order of $10^6$ per m.$^3$ per minute, indicating a thousand-fold concentration of radiophosphorus in the weed over that in the water. It is unfortunate that no quantitative data are available on the population of littoral plants in Linsley Pond, though, except at the south end, no extensive beds occur. The results just given suggest that in lakes in which there are wide expanses of littoral vegetation, this vegetation competes with the phytoplankton for phosphorus. Such competition is of course likely to be most severe during the growth period of the plants from April to June, and may conceivably account for phytoplankton minima often observed at the end of May or June.

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[4] Fisher, R. A., and Yates, F., Statistical Tables, London, 1938.